

**Linking plant traits and herbivory in grassland biodiversity-ecosystem  
functioning research**

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Submitted in partial fulfillment of the  
Requirements for the degree of  
Doctor of Philosophy  
in the Graduate School of Arts and Sciences  
COLUMBIA UNIVERSITY

2011

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## ABSTRACT

### LINKING PLANT TRAITS AND HERBIVORY IN GRASSLAND BIODIVERSITY- ECOSYSTEM FUNCTIONING RESEARCH

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Increased availability of data on morphological, physiological, and behavioral traits of species has improved understanding of the processes driving community assembly and the consequences of community disassembly for ecosystem functioning. In addition, there has also been a call for advancing the multitrophic view of biodiversity-ecosystem functioning. Here I propose a trait-based framework to merge plant-herbivore interactions with biodiversity-ecosystem function relationships. This framework links plant growth and defense strategies, herbivore feeding preferences, and primary production in terrestrial plant communities. I empirically tested these proposed linkages in laboratory and field experiments carried out in the understudied grasslands of Inner Mongolia, China. I found that a dominant generalist grasshopper *Oedaleus asiaticus* exhibits feeding preference for plants of high palatability when equally available, but in the field feeds on nearly any graminoid which is dominant. This behavior potentially allows subdominant plants to coexist, maintaining plant diversity.

To link feeding behavior to consequences for plant communities, I carried out detailed measurements of plant morphological and physiological traits in the field and experimentally manipulated grasshopper feeding intensity. Using a novel analytical tool, I found that plant communities in these grasslands exhibit high niche overlap, regardless

of intensity of herbivory by grasshoppers or sheep. This result indicates that environmental filtering structures these communities more than limiting similarity.

Extending the use of traits beyond the study system in Inner Mongolia, I test the how both functional and phylogenetic diversity explain the biodiversity effect on grassland ecosystem functioning. The promise for merging tools from evolutionary biology and functional ecology is great, as these diversity metrics provide superior explanatory power in a meta-analysis of biodiversity experiments. Future work should be addressed at clarifying which functional traits are most strongly reflected in measures of phylogenetic diversity, including strategies of compensating for or avoiding herbivory.

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## ACKNOWLEDGMENTS

I owe many thanks to all who have supported my years of graduate studies. I am grateful to my advisor, Shahid Naeem for giving me the opportunity to freely pursue collaborations and research projects, as long as I eventually worked on my thesis. He also constantly pushed me to see the big picture beyond every data point, a habit I will strive to maintain. The extended Naeem Lab has given invaluable inspiration and feedback on many of the project I undertook during my time at E3B, and has provided a supportive research and writing environment. María Uriarte opened my eyes to new tools for statistical analysis, and to the intriguing puzzles of secondary forests in Puerto Rico. Jim Elser, Oswald Schmitz, and Kevin Griffin have been generous with their time in helping me develop and refine this dissertation.

Yongfei Bai, director of the Inner Mongolian Grassland Ecosystem Research Station, provided me with a home base during my research summers, and was generous in giving me access to long-term field sites, data, and other resources. Many friends helped make those summers fruitful and productive, especially Georgia Hart, Jing Xing, Arianne Cease, Philipp Schönbach, Nicole Faneslow, Katerin Müller, Lü Xiaotao, Wei Cunzheng, Zhang Guangming, and Guo Jingguo.

My parents have always been eager to hear about each milestone reached , which has helped push me forward. With my partner and co-conspirator Mika at my side, every step has been twice as much fun.

## CHAPTER 1. INCORPORATING HERBIVORY AND PLANT DEFENSE

### STRATEGIES INTO BIODIVERSITY-ECOSYSTEM FUNCTIONING RESEARCH

#### Introduction

The last two decades have witnessed an explosion of research demonstrating that the diversity of a biota can influence ecosystem processes via a number of mechanisms (Hooper *et al.* 2005; Spehn *et al.* 2005; Balvanera *et al.* 2006; Cardinale *et al.* 2006). These studies were initially almost exclusively conducted in temperate grasslands, and the vast majority of experimental and observation work on biodiversity-ecosystem functioning (BEF) research remains focused on plant communities. Given that one of the ultimate goals of biodiversity-ecosystem functioning research is to inform biodiversity conservation for the better provisioning of ecosystem services (Naeem *et al.* 2009), there is a need to move beyond a purely plant-centric approach to BEF research. Despite the fact that herbivory strongly influences plant population composition and dynamics and that plant defenses influence herbivore behavior, relatively little attention has been paid to how feedbacks between herbivore and plant community dynamics fit into the BEF framework. This deficiency is evident even for terrestrial ecosystems like grasslands which have been a primary focus of BEF research. This review focuses on this issue of how herbivory influences the relationship between plant biodiversity and terrestrial ecosystem function.

Recently there have been calls to incorporate a food-web perspective in BEF research (Duffy *et al.* 2007; Reiss *et al.* 2009), with recent evidence that biodiversity effects cascade up trophic levels with a predictable level of attenuation (Scherber *et al.*

2010). However, there have still been few efforts to incorporate plant-herbivore interactions, in particular how herbivore behavior shapes plant communities and how variation in plant defense strategies may alter BEF relationships. As researchers move toward a more nuanced understanding of how biodiversity loss is altering ecosystems, it will no longer be sufficient to ignore how trophic interactions shape plant communities and thus the ecosystems that provide supporting, regulating, and provisioning services to society.

This review addresses three aspects of how plant-herbivore interactions affect biodiversity-ecosystem functioning relationships. Herbivory in biodiversity-ecosystem functioning research fits within a larger program of ecological research, ranging from the dynamics of herbivore interactions to plant-soil interactions (Fig. 1), but for the purpose of this review I focus on three elements of this research framework. First, I describe the current evidence for top-down control of plant communities and consequences for plant diversity ecosystem processes. Second, I detail how plant growth defense strategies differ across species and how this diversity is likely to alter biodiversity-ecosystem functioning relationships. Finally, I discuss the consequences of plant-herbivore interactions for different aspects of plant community diversity, in particular the relative importance of functional and phylogenetic diversity metrics as predictors of ecosystem functioning.

### **Top-down control of plant communities in a BEF framework**

Ecology has a long history of examining how the abundance of a given trophic level is determined by the abundances and number of connected trophic levels (Hairston *et al.* 1960; Oksanen *et al.* 1981), as well as extensive research into how plant defense

strategies vary within and across species (Fritz & Simms 1992). Yet while biodiversity-ecosystem function (BEF) research initially focused on complex food webs, including several trophic levels (Naeem *et al.* 1995; Naeem & Li 1997), the field soon narrowed its focus to examining the sign and shape of diversity-productivity relationships within the producer trophic level. This line of research has uncovered a robust positive, saturating relationship between species diversity and ecosystem properties ranging from net primary production to invasion resistance (Balvanera *et al.* 2006). However, research across trophic levels has been limited, strongly biased towards microbial and aquatic systems, with a notable lack of research on terrestrial plant-herbivore interactions.

In order to examine how terrestrial plant-herbivore interactions may affect BEF relationships, it is worth first reviewing evidence from multitrophic BEF research in general. Focusing within producer, herbivore, decomposer, and predator trophic levels, BEF relationships are consistently strong, in both terrestrial and aquatic systems (Cardinale *et al.* 2006). However, to date no comprehensive review of multitrophic BEF research has been conducted. As a first step, I compiled a list of studies which addressed the question of how diversity manipulation at one trophic level alters the BEF relationship at another level. These references were located from ISI Web of Science using the search term "biodiv\* AND ecosystem\* AND (function\* OR proces\*) AND (herbivor\* OR predator\* OR consumer\*)", as well as from references cited in the most comprehensive current review (Duffy *et al.* 2007). From these, I selected only those with experimental manipulations of diversity, and where biodiversity-ecosystem functioning relationships at an adjacent trophic level were assessed. This selection process resulted in 41 studies from 1997-2010, 19 terrestrial and 22 aquatic (Table 1.1). This summary

shows that while multitrophic BEF research had pursued more in aquatic systems initially, now the research effort is more evenly divided among terrestrial and aquatic systems. Niche complementarity has been identified as the mechanism driving the observed BEF relationships in just over half the cases for both aquatic systems (12 / 20 studies for which a mechanism was proposed), and terrestrial systems (7 / 13 studies).

The central advance made by BEF research has been that species composition can control ecosystem processes, by niche partitioning or by selection effects. This advance is based principally of findings from studies of BEF relationships in terrestrial primary producer communities, especially grassland plants. While such study systems are tractable and easily manipulated, this focus on grasslands and primary production has been acknowledged to have limited progress in this field (Loreau *et al.* 2001; Petchey *et al.* 2004a). However, in multitrophic BEF empirical research, this trend is reversed: progress has mostly been made in manipulating consumer diversity in aquatic systems and soil and microbial systems, with very limited work in terrestrial systems. This difference in the experimental focus is matched by a difference in conclusions about what mechanism drives BEF relationships: niche partitioning effects are commonly seen at the producer level, but selection effects dominate at consumer levels (Duffy 2002; Balvanera *et al.* 2006; Cardinale *et al.* 2006; Duffy *et al.* 2007). Aquatic (primarily marine mesocosm studies) and soil food web studies diverge in their manipulations of diversity from terrestrial (primarily grassland) studies, with terrestrial studies almost always only manipulating the producer trophic level. In addition, the mechanism driving the diversity-function relationships across trophic levels is most often identified as selection effects,

while niche complementarity effects predominate in terrestrial producer-manipulation BEF experiments.

When terrestrial ecologists have explicitly considered trophic structure in BEF experiments, they have done so almost exclusively by examining herbivore abundance as a function of plant richness, rather than by examining how herbivores alter producer diversity and abundances, and therefore ecosystem function. This diverges from the top-down approach considered by aquatic BEF experiments, but provides some indications of how top-down influences of herbivores may influence terrestrial BEF relationships. In the most common approach, researchers have used existing grassland BEF experiments (Koricheva *et al.* 2000; Scherber *et al.* 2006) or natural plant diversity gradients (Moretti *et al.* 2006; Unsicker *et al.* 2006; Harvey *et al.* 2008; Wenninger & Inouye 2008) to ask whether herbivore abundance and net consumption of plants decreases as plant species increases. For example, Koricheva *et al.* (2000) surveyed arthropod diversity in two combinatorial grassland BEF experiments in Sweden and Switzerland, finding that generalist herbivores significantly decreased in abundance with plant species richness. Similar results were found in a review of many studies of arthropod herbivory in forests, where tree diversity reduced herbivory significantly, presumably because herbivore abundances were decreased (Jactel & Brockerhoff 2007). Recent work integrating across many measures of arthropod, small mammals, nematode, and other trophic level diversity at a single BEF experiment has shown that manipulations of plant diversity have cascading "bottom-up" effects, with herbivore abundance and diversity declining with reduced plant diversity (Scherber *et al.* 2010).

These outcomes of declining herbivore abundance with increased plant diversity provide some support for the concept of associational resistance (Callaway 1995). In associational resistance, producers benefit from having a diversity of neighbors with varying palatabilities, or varying apparency, where greater diversity decreases consumer efficiency by increasing the search time it takes specialist herbivores to find their preferred producer species (Chew & Courtney 1991; Milchunas & Noy-Meir 2002). Associational resistance has also been found in many manipulations of aquatic producer diversity, where susceptibility to herbivory was reduced by higher producer richness (Hillebrand & Cardinale 2004; Long *et al.* 2007); this is a rare case of agreement between terrestrial and aquatic BEF research. For either associational resistance or apparency effects, species richness *per se* is secondary in importance to variation in plant resistance and tolerance to herbivory, an important point which highlights the importance of focusing on traits of species rather than simply species richness in BEF research.

While this review focuses on the effects of herbivory and plant defense strategies in BEF research, it is worth noting that manipulations of consumer or predator diversity in terrestrial trophic BEF experiments remain nearly nonexistent. One of the only examples of this type of study comes from Schmitz (2006), who manipulated presence of predatory spiders in field mesocosms, showing that predator effects on herbivorous arthropods altered plant diversity and thus nitrogen cycling and light penetration to the soil. Previous studies have shown increased nutrient cycling due to grasshopper herbivory, but only through changes in leaf litter abundance (Belovsky & Slade 2000), not plant species composition. Schmitz (2008) extended this work to demonstrate that not just predator presence but the hunting strategy controlled this tri-trophic relationship. In

particular, variation in hunting mode of the predator altered herbivore behavior, leading to dramatic reductions in both the abundance of a dominant plant species and plant diversity, with corresponding changes in aboveground net primary production, nitrogen mineralization, and litter carbon:nitrogen content (Schmitz 2009). Similarly, Steffan & Snyder (2010) found that greater diversity of predators, even when consumption of prey was prevented, lead to greater plant biomass via greater disruption of herbivory. Little comparable work linking individual behavior, diversity, and ecosystem functioning exists in aquatic systems for comparison, and more work in terrestrial systems is needed to extend the generality of this work.

## **Plant growth and defense strategies in BEF**

In assessing top-down effects of herbivores on plant communities, a crucial aspect is the strategies employed by plants in resisting herbivory. Producers are not necessarily passive casualties of herbivory. Producers have a variety of strategies within the broad categories of resistance and tolerance to herbivory (Fritz & Simms 1992). Strategies to resist herbivory range from mechanical defenses of spines and leaf toughness (Lucas et al. 2000), to indirect defenses such as reduced nutritional quality (Coley et al. 1985). Plants can also employ highly targeted indirect defenses, for example where root herbivory on cotton leads to increased extra-floral nectary production, recruiting predators to feed on the herbivore (Wackers & Bezemer 2003). There are many examples of such strategies and a large, well-developed literature on when and how plants employ herbivore defense strategies and how they affect fitness (Mattson 1980; Herms & Mattson 1992).



These strategies have generally been conceived of as falling along a trade-off axis where plants vary investment in growth to investment in defense. From the perspective of herbivores, this variation in plant strategies places feeding preferences in a general framework of balancing the search for nutrition from fast-growing plant tissues and avoiding toxicity from well-defended plant tissues. This search for nutrition and avoidance of toxicity clearly has the potential to alter plant community structure, and thereby alter ecosystem functioning.

Herbivores are known to alter their feeding patterns in order to address nutrient deficiencies (Raubenheimer & Simpson 1997), leading in some cases to generalist herbivores partitioning plant resources into nutritional niches, promoting coexistence (Behmer & Joern 2008). The emphasis on nutritional preferences of herbivores leads naturally to examinations of the plant nutrients which affect plant growth, in particular nitrogen or protein content. For example, two generalist vole species were found to have similar food preferences across 20 plant species, both preferring plants which had high regrowth ability and nitrogen content, even though these plant varied broadly in morphology and defensive compounds (Hjalten et al. 1996). Such evidence supports the notion that plant growth strategies are linked with palatability to herbivores, with particularly strong evidence for high nitrogen, fast-growing or regrowing plant species being more highly preferred by ungulate herbivores (Bryant *et al.* 1989; Augustine & McNaughton 1998).

The converse of herbivore preferences for fast-growing, high nutrient content plants is avoidance of toxic plant defenses. In some cases, evidence shows that avoidance of defensive compounds clearly predict food preferences of herbivores, and nutrient content

of plants does not (Bryant & Kuropat 1980). Other evidence shows that the effectiveness of a defensive compound, tannic acid, deterred feeding by an insect herbivore only in certain experimental diets, indicating a complex interaction between nutrition, toxicity, and feeding (Behmer et al. 2002). Promising work from a mathematical model has shown that in the absence of variation in palatability, herbivory can promote plant species coexistence, but when palatabilities vary, herbivores shift plant communities towards dominance by a few unpalatable species (Feng et al. 2009).

This variation in growth and defense strategies, and the resulting herbivore feeding preferences, presents an opportunity for a more trophically-explicit BEF research framework. As an example of how to incorporate consideration of plant defenses into trophic BEF research, Chapman and colleagues (2003) demonstrated that pinyon pines (*Pinus edulis*) vary genetically in susceptibility to herbivory from a scale insect and a stem-borer, and that litter from more susceptible trees decomposed faster, "super-charging" the nutrient acceleration effect of herbivores in this system. The amount of intraspecific diversity in resistance to herbivory therefore influenced a key ecosystem process.

Theoretical studies on the impact of variation in growth-defense tradeoffs for communities have followed the tradition established by May (1973), focusing on the consequences of evolution of defense strategies for the relationship between species richness and community stability. Such work has been based on Lotka-Volterra models with the tradeoff represented by inverse relationship between changes in interaction coefficients  $\alpha$  and population growth rates  $r$ , such that an innovation which reduces the negative effect of another species also reduces population growth rates. Such work has

demonstrated that increased ability to allocate resources to defense can destabilize communities (Abrams 2000), but in low diversity communities can promote stabilization of both plant and herbivore populations (Loeuille 2010). Recent empirical evidence in plankton communities shows that increased stability as a result of stronger allocation to defense mechanisms is possible (Aránguiz-Acuña *et al.* 2010). Theoretical work has not been advanced to link the growth-defense tradeoff in plants to ecosystem functioning. These examples demonstrate that future work remains necessary incorporating plant defense strategies in trophic BEF research.

In order to envision how to more generally incorporate variation in plant defense strategies into BEF research, I make several simplifying assumptions. First, I assume that the major tradeoff axis in plant defense strategies is between growth and defense at the level of the individual organism; thus, variation in reproductive investment is not directly accounted for here. Plants often exhibit this regrowth-defense tradeoff (van der Meijden *et al.* 1988), and some plants regrow so successfully from grazing by ungulate herbivores that it has been proposed that there is a class of plants for which grazing stimulates overcompensatory growth (McNaughton 1983). However, later work demonstrated that such overcompensation is rare and occurs mainly when interspecific competition is weak (Belsky 1986). Regardless, it is well established that there is a continuum of responses to herbivory, along the regrowth-defense axis (Herms & Mattson 1992; Alward & Joern 1993).

Second, I assume that this tradeoff is strongest between species, rather than between individuals of a species, such that variation in species composition will alter the balance of growth and defense strategies in the community. This assumption means that

communities may be composed mainly of "defenders", which grow slowly and lose little biomass to herbivory (Fig. 2, Group I), mainly of "regrowers", which may lose biomass readily to herbivory but recover quickly (Group II), or a wide range spanning both types (Group III). The consequences of variation in these strategies can then be envisioned.

For a community of "defenders", the relationship between plant diversity and primary production may be steeper than expected when compared to a null community, if the moderate investment in defense mechanisms means that little biomass is removed by herbivory. Therefore any gains in primary production attributable to an increase in diversity (either via selection or complementarity) are kept. Conversely, under high herbivory a great degree of resources are shifted to defense, not growth, making the BEF relationship weaker than expected (Fig. 1.3, top panels). Communities composed of "regrowers" would show the opposite pattern, with BEF relationships weaker than expected under minimal herbivory, but greater than expected under high herbivory. The latter would result from compensatory or even overcompensatory growth being the primary strategy for responding to herbivory (Fig. 2, middle panels). Finally, considering communities composed of a range of defense strategies, there is no clear *a priori* hypothesis for how herbivory should alter the biodiversity-ecosystem functioning relationship (Fig. 1.2, bottom panels).

Herbivore feeding preferences depend in part on the balance between nutrition and toxicity of plant tissues. While debate remains about how to properly place nutritional ecology in a theoretical framework (Raubenheimer et al. 2009), it is clear that herbivores can structure plant communities, thereby altering the producer diversity and presumably ecosystem functioning. Ideally, these hypotheses could be tested by

manipulating plant community composition to create experimental communities ranging from pure "defenders", to a mix, to pure "growers". By manipulating species richness within each of these mixes, and overlaying that with intensity of herbivory, the shape of these relationships could be elucidated. To date no field experiment has been designed that fits these requirements. However, in the following chapters I describe results of a series of observational and experimental studies to first clarify what strategies exist in grassland plant communities of Inner Mongolia, China, and also examine how intensity of herbivory shapes the plant community assembly. An ideal next step would be to design an experiment based on these studies with a range of community, varying the identity and richness of species within each community type.

### **The B in BEF: Diversity measurement in herbivore-structured plant communities**

Extending the plant-herbivore interactions to the ecosystem level is the goal of this review. In addition to incorporating top-down effects of herbivores on plant communities in combination with variation in plant strategies for dealing with herbivory, a complete trophic BEF research agenda must also be explicitly about how to connect plant diversity with ecosystem functioning. In particular, which facet of biodiversity most strongly influences ecosystem functioning remains a subject of debate. The limitations of simply counting taxonomic units has long been recognized (e.g., Wilsey 2005), and in recent years a flurry of new metrics for assessing biodiversity have been proposed, largely using some combination of species traits to calculate a measure of functional diversity (Petchey et al. 2009). Other noteworthy metrics include the functional group

richness and summarizing the evolutionary history of a community using a measure of phylogenetic diversity. For plant communities under herbivory, how should these different metrics perform as predictors of biodiversity-ecosystem functioning relationships? This question is relevant because if the top-down effects of herbivores strongly structure plant communities, then a diversity measure which reflects variation in plant defense strategies may be more relevant to ecosystem functioning than species richness alone.

A tradition from the earliest BEF studies has been to focus on species richness, both in experiments and observational studies. Early criticism from other ecologists has focused on the fact that as in experiments, the higher species richness levels were more likely to include the species with the greatest influence on ecosystem processes by chance alone (Huston 1997; Wardle 1999). Thus, rather than greater number of species resulting in more effective partitioning of resources (niche complementarity), this sampling or selection effect shows that particular species or species combinations can drive ecosystem processes. Selection effects are common in BEF experiments, particularly for consumer or predator diversity effects (Cardinale et al. 2006), although strong evidence for niche complementarity exists (Hooper et al. 2005), particularly for producer diversity effects (Spehn et al. 2005).

The predominance of selection effects in multitrophic BEF studies (Table 1) may be resolved in part by appropriate choice of a biodiversity metric, functional diversity in particular. Many researchers accept that the selection effect can be a legitimate biological mechanism by which reductions in biodiversity are likely to impair ecosystem functioning (Loreau 2000; Hector *et al.* 2002; Fox 2005), acting on community

composition at larger spatial scales (Loreau et al. 2001) or longer temporal scales (Pacala & Tilman 2001) than niche complementarity. A growing focus on the diversity and values of functional traits that influence ecosystem functioning, as a tool for explaining the role of organisms in ecosystems and the ecological impacts of their loss (Petchey & Gaston 2006).

For plant communities structured by herbivores, functional traits such as specific leaf area (correlated with leaf toughness, a mechanical defense against herbivory) can have reduced range (Díaz et al. 2001). Reduction in the diversity of this trait would be revealed by a metric of functional diversity, but not species richness. In addition, the presence of particular species which could thrive under herbivory would likely have a unique combination of functional traits, meaning that a functional diversity metric would capture this increase in diversity in ways relevant to ecosystem functioning.

Phylogenetic diversity has recently been proposed to be a stronger predictor of grassland biodiversity-ecosystem functioning relationships (Cadotte et al. 2008). In that work, Cadotte et al. (2008) conducted a meta-analysis of biodiversity-ecosystem functioning studies, finding that phylogenetic diversity (PD) predicted the biodiversity effect on grassland biomass accumulation stronger than species richness or functional group richness. The authors suggested evolutionary diversification has generated trait diversification for these species, which in turn may result in greater niche complementarity. This suggests that PD (the distinct evolutionary history in a community) can be an effective proxy for functional diversity, the functional trait distinctiveness in a community). If variation in plant defense strategies is highly tied to

variation in evolutionary history, then PD will be a strong predictor of biodiversity-ecosystem function relationships for plant communities under herbivory.

The use of phylogenetic diversity to predict ecosystem function assumes phylogeny accurately represents functional differences (Maherali & Klironomos 2007). This assumption will only be true if there is a strong phylogenetic signal in the ecological relationships between species. Unrelated species can easily exhibit similar functional trait phenotypes, particularly traits related to resource capture like height and photosynthetic rate. However, for traits reflecting plant defense against herbivory there is a vast literature on coevolutionary relationships between plants and their herbivores (Karban & Agrawal 2002; Agrawal *et al.* 2006). However, such studies have historically focused on particular pairs of plant and insect herbivore species, rather than examining how defense traits are predicted by phylogeny across a plant community. This remains an unanswered question.

An additional consideration with respect to phylogenetic diversity is that knowledge of which traits are important to ecosystem functioning and access to high-quality trait data are lacking for most species and ecosystem functions of interest; PD could be quite valuable as a proxy for FD. Data on plant ecophysiology and life history are copious, but data on defense strategies across communities have not been compiled. Research in grassland communities has underscored the importance of leaf traits for net primary production, in particular leaf mass per unit area (Garnier *et al.* 2004; McIntyre 2008), leaf percent nitrogen (Kahmen *et al.* 2006), belowground traits such as root thickness (Craine *et al.* 2002) and nitrogen-fixation (Lee *et al.* 2003), and whole-plant traits such as height (Díaz *et al.* 2007) in controlling ecosystem processes. In a meta-



analysis, the functional diversity of these traits was a better predictor of biodiversity effects on grassland primary production than phylogenetic diversity, although both were dramatic improvements over species richness or functional group richness (see Chapter 4). However, little phylogenetic signal was detected for variation in any of those traits, suggesting that it must be variation in other traits related to plant diversity effects, perhaps variation in plant defense strategies, which gives phylogenetic diversity such high predictive power.

## **Conclusions**

In order to better understand how continuing species losses will affect the world's ecosystems, biodiversity-ecosystem functioning research should revisit its origins as a multitrophic research framework. For terrestrial ecosystems, a long tradition of plant-herbivore interaction work at the community level provides a basis for researchers to incorporate top-down effects of herbivores on plant communities. Parallel research into the causes and consequences of herbivore feeding preferences, notably on variation in plant strategies along a growth-defense axis, provide a starting place for this effort. Finally, in order to extend the work to the ecosystem scale, appropriate measures of plant biodiversity need to be considered.

Table 1.1. Selected biodiversity-ecosystem functioning experiments across trophic levels.

Diversity-response relationships are summarized according to the aims of the study.

Proposed mechanism (Mech) summarizes what the authors concluded drove the observed relationship, if any: N = niche, S = selection effect.

<i>Study</i>	<i>System</i>	<i>Tr. levs</i>	<i>Trophic level manipulated</i>	<i>Response</i>	<i>Diversity- response relationship</i>	<i>Mech</i>
Aoki 2003	Freshwater lake	3	None	Producer biomass	Positive linear	
Jaschinski et al 2009	Freshwater mesocosm	2	Consumer (mesograzers)	Producer diversity and biomass	Positive/Idiosyncratic	N
Downing 2005	Freshwater mesocosm	3	Producers (macrophytes), consumers (gastropods), predators (macroinvertebrates)	Producer and consumer abundance	Idiosyncratic	S
Steiner 2001	Freshwater microcosm	2	Producer (algae), consumer (crustaceans)	Producer and consumer biomass	Negative (prod. on cons., vice versa)	S
Naeem et al. 2000	Freshwater microcosm	2	Producer (algae), decomposer (bacteria)	Resource use	Positive	N
Srivastava & Bell 2009	Freshwater microcosm	4	Consumer (detritivores)	Predator and other consumer diversity and abundance	Positive	N
Naeem & Li 1997	Freshwater microcosm	6	Producer, decomposer, consumer	Producer biomass	Positive linear	N
Bastian et al. 2008	Freshwater stream	2	Leaf litter, decomposer (leaf shredding larvae)	Decomposition	Idiosyncratic	S
Jonsson & Malmqvist 2000	Freshwater stream	2	Decomposer (leaf shredders)	Decomposition	Positive linear	N
Raberg & Kautsky 2007	Marine mesocosms	2	Consumer (invertebrates)	Producer biomass and diversity	Idiosyncratic	S
Duffy et al. 2001	Marine mesocosm	2	Grazer	Producer biomass consumed	Idiosyncratic	S
O'Gorman et al. 2008	Marine food web	3	Predator (fish)	1° and 2° Consumer abundance	Negative (2°), positive (2°)	
Bruno & O'Connor 2005	Marine food web	3	Predator (crabs, fish)	Producer biomass and diversity	Negative	N+S
Douglass et al. 2008	Marine food web	3	Producers consumer, predator	Abundances of each level	Negative (on graz), positive (on prod.)	N+S
Griffin et al. 2008	Marine food web	3	Predator (crabs)	Resource capture rate	Positive	N
Bruno et al. 2008	Marine food web mesocosm	2	Producer (algae), consumer (invertebrates, fish)	Producer and consumer biomass	Negative (cons. on prod., vice versa)	N+S
Burkpile & Hay 2008	Marine food web mesocosm	2	Consumer (fish, urchin)	Producer abundance	Negative	N
Byrnes et al. 2006	Marine food web mesocosm	3	Predator (crabs)	Producer (kelp) biomass	Positive	N
O'Connor & Bruno 2009	Marine foodweb	3	Predator (crabs, shrimp, fish)	Prey and producer abundance	Idiosyncratic	S

Altieri et al. 2009	Marine mesocosm	2	Consumer (snail herbivore)	Producer (seaweed) diversity and productivity	Positive	N
Gamfeldt et al. 2005	Marine microbial microcosm	2	Producer (algae), consumer (ciliates)	Producer biomass remaining, consumer biomass	Positive linear	S
Moothri et al. 2008	Marine microbial microcosms	2	Consumer (ciliates)	Consumer biovolume	Positive linear	S
Jactel et al. 2007	Forest	2	None	Consumer abundance	Negative	S
Schuldt et al. 2010	Forest	2	None	Consumer diversity and abundance	Positive	N
Philpott et al. 2009	Forest	3	Predator (birds)	Prey diversity and abundance	Positive	N
Unsicker et al. 2006	Grassland	2	None	Consumer abundance	Negative	
Hartley & Jones 2003	Grassland	2	None	Consumer species richness	Positive curvilinear	
Moretti et al. 2006	Grassland	2	None	Consumer abundance and composition	Positive (abundance)	
Scherber et al. 2006	Grassland	2	Producer (plants), consumer (arthropod)	Producer abundance	Positive	S
Wenniger & Inouye 2008	Grassland	2	Producer (plants)	Consumer abundance and composition	Idiosyncratic	
Snyder et al. 2006	Grassland	3	Predator (spiders)	Consumer (aphid) abundance	Negative	N
Haddad et al. 2009	Grassland	3	Producer (plants)	Consumer diversity and abundance	Positive	N
Koriecheva et al. 2000	Grassland	3	Producer (plants)	1° and 2° Consumer abundance	Idiosyncratic	
Naeem et al. 2005	Grassland mesocosm	4	Producer (plants), consumer (inverts), decomposer, parasitoid	Gas exchange, decomposition, producer biomass	Positive (respiration, production)	N
Griffiths 2000	Grassland soil	3	Decomposer (bacteria), consumer (protazoa), predator (nematodes)	Total microbial biomass, soil N	Idiosyncratic	S
Steffan & Snyder 2010	Plant	3	Predator (wasps)	Producer biomass	Positive	N
Wardle et al. 1999	Soil food web	2	Producer (plants)	Consumer abundance and composition	Idiosyncratic	S
Scheu et al. 2002	Soil food web microcosm	2	Consumer (earthworms)	Producer abundance	Negative	S
Mikola & Setälä 1998	Soil food web microcosm	3	Microbial, consumer (nematodes), predator (nematodes)	Microbial biomass and respiration	Positive/idiosyncratic	S
Deacon et al. 2006	Soil microcosm	2	Decomposer (fungi)	Litter abundance	Idiosyncratic	N+S
Scherber et al. 2010	Grassland	4	Producer (plants)	Consumer, predator, parasitoid, and omnivore abundance	Positive	

Figure 1.1. Conceptual schematic of how physiological tradeoffs in herbivores and plants affect herbivore and plant communities in terms of biodiversity, the interaction between herbivores and plants, and the resulting stocks of herbivore, plant, and nutrient mass. The foci of this review are in bold: 1. top-down effects of herbivore communities on plant biodiversity, 2. role of tradeoffs between growth and defense affecting plant communities under herbivory, and 3. how the biodiversity of resulting plant communities affects ecosystem functioning in terms of biomass production.

Figure 1.2. Three hypothetical cases of variation along a tradeoff of two general plant functional traits, defense and growth. I. All species well-defended and slow-growing; II. all species poorly-defended and fast-growing; III. wide range of allocation to defense and growth.

Figure 1.3. Hypotheses of how variation in plant defense and herbivory interact in determining biodiversity-ecosystem function (BEF) relationships. Light curves show typical BEF relationships without considering the effect of either plant defense strategy or herbivory. Black curves show hypotheses for how BEF relationships may be modified under the three cases of variation in plant defense strategy presented in Fig. 1 and under either low or high herbivory. For case III, no a priori hypothesis is immediately clear. All hypotheses make the simplifying assumptions of constant resource supply, no interactions among herbivores, only generalist herbivory, and no predators. See text for details.

Figure 1.1. Conceptual schematic of growth-defense tradeoffs affecting BEF relationships.

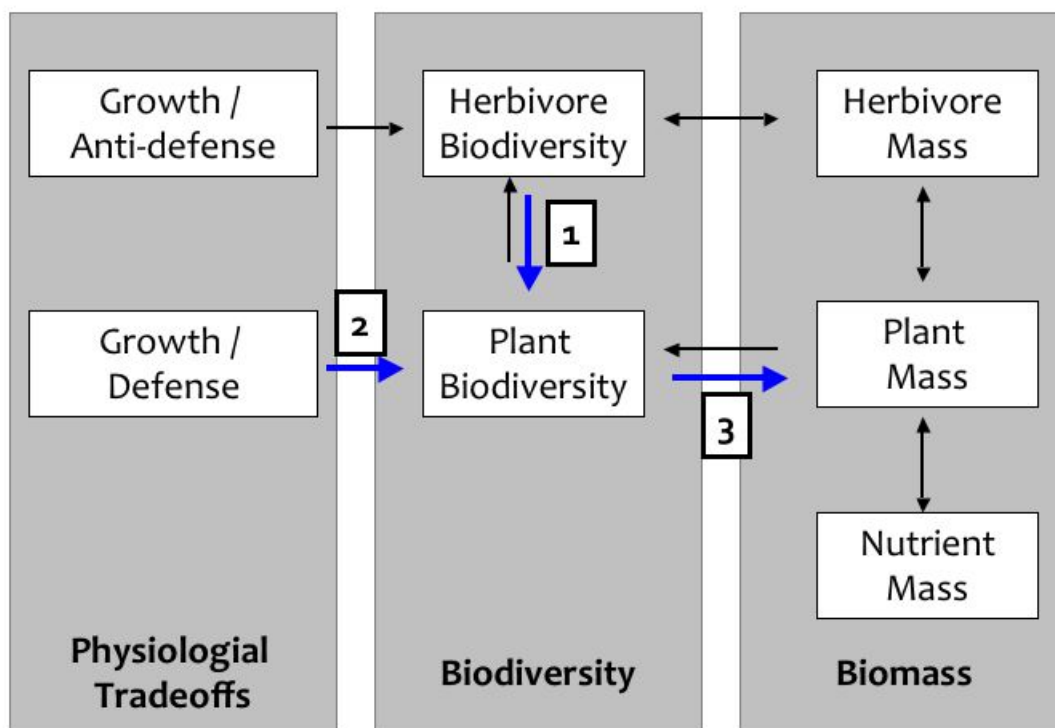


Figure 1.2. Hypothetical relationships between growth and defense.

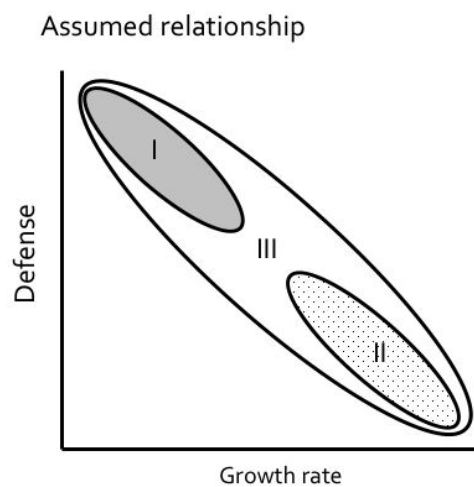
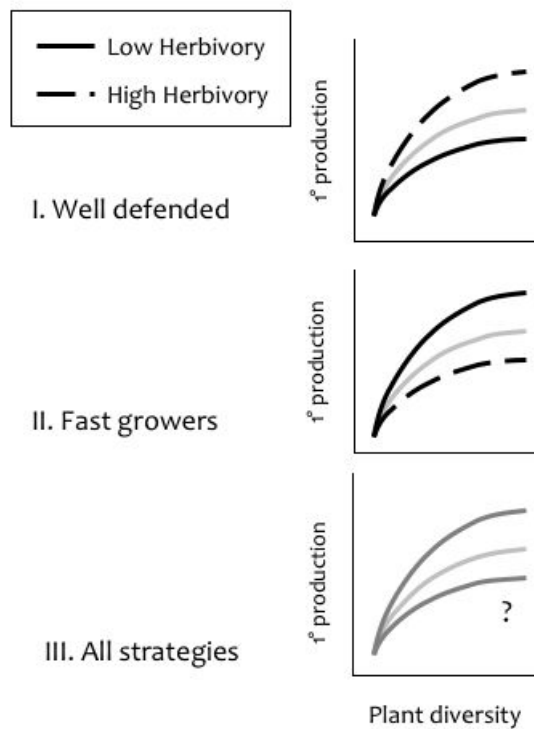


Figure 1.3. Hypotheses linking growth-defense tradeoffs, herbivory, and plant community biomass production.



## CHAPTER 2. FORAGING BEHAVIOR OF A GENERALIST GRASSHOPPER, *OEDALEUS ASIATICUS*, IN RESPONSE TO PLANT COMMUNITY COMPOSITION AND PLANT TRAITS

### Summary

Integrating herbivory into a biodiversity-ecosystem functioning research framework requires assessing how top-down effects of herbivory may play out for plant communities, namely assessing the feeding behavior of the key herbivores. Here, I use a series of experiments to assess 1. the feeding preferences of a dominant grasshopper, *Oedaleus asiaticus* on grassland plant species in Inner Mongolia, China; 2. observed feeding behavior in the field for this grasshopper; and 3. how these preferences and behavior relate to plant nutrient and antiherbivore characteristics.

I found that in controlled laboratory settings the grasshopper *Oedaleus asiaticus* has a strong preference for a thin-leaved, short-statured plant, *Cleistogenes squarrosa*. However, the preferences observed in the lab were not detectable in the field. Increases in leaf silica of the co-dominant rhizomegrass *Leymus chinensis* and decreases in leaf silica of the co-dominant bunchgrass *Stipa grandis* in response to herbivory, as well as the strong avoidance of the fairly N-rich grass *Achnatherum sibericum*, demonstrated that antiherbivore defenses may explain feeding preferences of grasshopper in this grassland system. Extending this work will help to understand the top-down effects of herbivory on grasslands, and integrate herbivory more fully into research on terrestrial biodiversity-ecosystem functioning relationship.

## Introduction

Translating herbivore behavior to ecosystem functioning requires an understanding of the factors shaping foraging decisions in herbivores. That is, understanding the effects of individual herbivores on plant communities at the local scale is the basis for more broadly understanding how herbivores shape ecosystem functioning. Research frameworks for investigating factors shaping the decisions of individual herbivores fit within the field of nutritional ecology, which includes the geometric and ecological stoichiometric frameworks (Raubenheimer *et al.* 2009). These research frameworks differ in their details, but all seek to relate herbivore feeding behavior to the search for nutrients and/or the avoidance of toxic compounds.

In order to address the question of how herbivore behavior shapes plant community composition and structure, I investigated the feeding preferences of the band-winged grasshopper, *Oedaleus asiaticus*, in Inner Mongolia, China. This research asks the related questions: What are the feeding preferences of a dominant generalist grasshopper in Inner Mongolia? How do feeding preferences assessed in controlled settings compare to feeding behavior observed in the field? And how do nutritional and toxic components of the dominant plant species related to feeding preferences of *O. asiaticus*?

*O. asiaticus* is a large and common grasshopper in Inner Mongolia, typically peaking in density in mid-July (Kang & Chen 1992). This species is considered a serious economic pest and is a graminivorous generalist which separates its niche from the forbivorous and omnivorous grasshopper species with which it coexists (Kang & Chen 1994). Substantial research efforts have been directed at understanding grasshopper



community and population dynamics at this site, but there has been surprisingly little investigation into the feeding preferences of *O. asiaticus* in the field, a notable omission given the importance of this species in the Inner Mongolian grasslands. *O. asiaticus*

To address these research questions, I conducted three experiments. First, I evaluated feeding preferences of *O. asiaticus* in laboratory settings, provisioning plant material from two species at a time out of a pool of six common species. Second, I observed feeding behavior of *O. asiaticus* individuals in a range of plant communities in the field, at both immature and mature life stages. Third, I assessed the degree of investment in chemical defenses of selected common plant species, focusing on investment in silica in leaf tissues, under conditions of feeding by *O. asiaticus* and experimental clipping.

In order to understand the feeding preferences of *O. asiaticus*, determining the nutrient and toxin concentrations of key food items is a crucial step. In response to graminivorous grasshoppers like *O. asiaticus*, plants may demonstrate a range of responses along the growth/defense tradeoff, which in turn may determine feeding preferences. Grasses have been shown to employ both phenolics (Rhoades 1985) and silica (Vicari & Bazely 1993) as defensive compounds in response to leaf-chewing herbivores. Silica has been shown to be an effective anti-herbivore compound, acting both as a mechanical defense against chewing (Massey *et al.* 2009) and reducing digestibility of leaf tissues by grasshoppers (Hunt *et al.* 2008). Silica often represents an inducible defense in which concentrations in plant tissues can increase after the plant is fed upon (Massey *et al.* 2007a), with greater concentrations observed in plant species which have lower growth rates (Massey *et al.* 2007b). In addition to avoiding defenses,

generalist grasshoppers also actively modify their intake of protein and carbohydrates to maintain a balanced nutrient intake (Behmer *et al.* 2002). Thus, both responses to plant defenses and plant quality shape herbivore feeding.

Compared to other grasshoppers at this site, *O. asiaticus* feeds on plants with a much wider range of height (Yan & Chen 1997). Previous work on feeding preferences of grasshoppers in Inner Mongolia has generally identified grasses as the preferred food items of *O. asiaticus* (Li & Chen 1985), but has not investigated relative preferences between these species, related these preferences to behavior in the field, or related these preferences to plant traits. In this study, in addition to examining grasshopper response to defenses and plant quality, I will examine relative preferences in relation to field behavior and plant traits.

## Methods

### *Study Site*

The study was carried out near the Inner Mongolia Grassland Research Station (43°38'N, 116°42'E) of the Institute of Botany in the Chinese Academy of Sciences. Located in the Xilin River catchment. This area has a continental, semi-arid climate, with mean annual precipitation of 334 mm and mean annual temperature of 0.7°C. The typical steppe ecosystem is dominated by C3 grasses, particularly the perennial rhizome grass *Leymus chinensis* and the perennial bunchgrass *Stipa grandis* (Bai *et al.* 2004). Given the relatively simple plant community structure, with fewer than 20 common plant species, this community is an ideal test case for examining how functional traits reflect the processes of habitat filtering or limiting similarity in structuring communities.

Two experiments were established to investigate the feeding preferences of the *O. asiaticus*. A third experiment was designed to evaluate the underlying mechanisms driving the feeding behavior in response to plant growth and defense strategies.

### ***Experiment 1: Pairwise preferences***

To establish the relative feeding preferences of *O. asiaticus*, I first addressed relative preferences in a pairwise comparison. I sought to establish the rank order and the relative preference for the dominant plant species. I assessed feeding preferences of *O. asiaticus* in an experiment where female grasshoppers were provided with small, equal samples of a pair of plant species, drawn from a pool of six species: *Achnatherum sibiricum*, *Agropyron cristatum*, *Carex duriuscula*, *Cleistogenes squarrosa*, *Leymus chinensis*, and *Stipa grandis*.

Ten replicates of the 15 pairwise combinations of 6 dominant species were run over three days in 2009. Leaf tissue was collected from the field site by clipping plants at the ground and transporting bundled plants of each species back to the field station. Fresh tissue was collected late afternoon on the day prior to the day of each trial. Sections of leaf tissue approximately 5 cm were cut and weighed on a fine-scale balance, then inserted vertically into a covered Petri dish with small holes drilled for plant samples. This experimental set-up ensured that equal portions of leaf tissue of each species were available, with freshness partially maintained by the water in the Petri dishes, and with plant samples arranged vertically and with equal distance from one another. Fifth-instar black morph female grasshoppers were collected from the field and maintained in cages

in the field near the field station. Grasshoppers were weighed and kept without food for 12 hours prior to the feeding trials to minimize the effect of prior feeding.

Analysis of feeding preference was carried out on a dry-mass basis. Concurrent with each set of feeding trials, five samples of fresh leaf tissue for each species of equal size to those used in the trials were weighed and placed in dishes adjacent to the feeding arenas. Then these samples were re-weighed at the end of the feeding trials (ca. 7 hrs). These weights were used to establish the average percent moisture for each species, allowing the conversion of the fresh leaf weights used in trials to dry weights. Relative feeding preference could then be assessed several ways. First, absolute total leaf mass consumed was assessed using a one-way analysis of variance (ANOVA) with focal species as the treatment variable. Second, relative preference of a focal species with respect to each comparison species was assessed using general linear models. Models were of the form  $Pref = f(Focal/Comparison)$ , where  $Pref$  is the relative preference of the focal species, expressed as the difference in the percent dry mass consumed of the focal species versus the comparison species:

$$Pref = \% DM Focal Sp Eaten - \% DM Comparison Sp Eaten$$

calculated for each experimental pair. The nested explanatory term allows comparison of the focal species relative to each of the five other comparison species. Finally, leaf trait data collected from the field (see Chapter 3) were used to test how feeding preferences relate to difference in leaf element concentrations, leaf thickness, and other traits considered influential for grasshopper feeding.

### ***Experiment 2: Feeding behavior in the field***

Using an experimental setup designed to test the effects of herbivory on plant community composition, I observed the behavior of 62 individual grasshoppers, both green (solitary) and black (gregarious) color morphs, in both immature and mature life stages. The two color morphs demonstrate significantly different metabolic rates and jumping ability (Cease et al. 2010), and may therefore be expected to have different effects on plant communities. Specifically, the larger, gregarious black morph may consume more and less selectively compared to the smaller, solitary green morph. Grasshoppers were placed in 0.25 m<sup>2</sup> x 1 m tall mesh cages in placed across a range of initial plant community compositions in June 2009. With one male and one female grasshopper in each cage, individuals could be tracked throughout the experiment. All activity was noted every minute for 30 minutes for a given cage, focusing on which plant species were eaten and how long each feeding bout lasted. A total of 87 observation hours were logged on a cage basis over eight days in July 2009. Observers were shielded by cloth coverings on the cage, and sat immobile for at least three minutes prior to observations, to minimize the disturbance on grasshopper behavior.

Analysis of feeding behavior in the field was carried out in several ways. First, total time eating, walking, or still was tallied by age (5<sup>th</sup> instar or adult) and sex. These were examined with respect to air temperature and precipitation data from a weather station approximately 250 m from the experimental cages. Second, total time eating any plant species was analyzed by age and sex across all cages. Third, to account for variation in plant community composition, general linear models with Poisson errors were used to assess time spent eating each of the six plant species. Poisson link was necessary due to

the abundance of zeros in the observational data; zero-inflated Poisson models did not perform significantly better. For plant species  $i$ , the models take the form

$$\text{Time Eating Species } i = f(\text{Stipa} + \text{Leymus} + \text{Agropyron} + \text{Carex} + \text{Cleistogenes})$$

where grasshopper feeding time is measured in minutes and relative abundance of the six commonly observed plant species are predictors. Models using the first two principal components of the entire community composition matrix were also assessed, but did not differ from the models used here, and were more difficult to interpret.

### ***Experiment 3: Feeding, clipping, and Si addition***

To assess interactions between feeding behavior and defense strategies I conducted an experiment in 2008 using the two dominant plant species, the bunchgrass *Leymus chinensis* and the needlegrass *Stipa grandis*, in the grassland communities surrounding the Inner Mongolia Grassland Ecosystem Research Station. Plants were transplanted from areas surrounding the field station into 4-L pots enclosed by mesh netting which was held up by circular wire frames 1 m tall. Three treatments were applied: (1) *O. asiaticus* where present or absent, (2) mechanical wounding which mimicked grasshopper feeding versus no wounding, and (3) silica added in the form of  $\text{SiOH}_4$  at 150 mg / L (Massey *et al.* 2007b) or no silica added. Twelve replicates were established for each treatment, for each species, for a total of 12 replicates x 2 species x 6 treatments = 144 pots. Silica solution was added four times over the course of the 57-day experiment. Mechanical wounding was accomplished by clipping half of all leaf material in a pot, and was carried out three times over the experiment. Grasshoppers were fourth-instar black morphs (see Chapter 3) of *O. asiaticus* collected from nearby fields, with one

male and one female added per grasshopper treatment pot. Enclosures were censused weekly, and additional grasshoppers were added as necessary. A total of nine grasshoppers of the original 96 (2 grasshopper treatments x 12 replicates x 2 grasshoppers per pot x 2 species) were replaced. Pots were watered twice weekly.

I additionally sampled leaf tissue of these two grasses in across an experimental sheep grazing intensity. Since 2005, a Sino-German collaboration has been experimentally manipulating sheep grazing intensity at the field scale near the IMGERS field site. This experiment, "Matter Fluxes in Grasslands of Inner Mongolia as Influenced by Stocking Rate" (MAGIM, <http://magim.net>), is aimed at elucidating how rangeland management affects grassland biotic and abiotic processes. Plants were sampled from two replicate fields with 0, 4.5, and 9 sheep per hectare. Full descriptions of the experiment, including details on how sheep densities were maintained in fields, can be found in Schönbach et al. (2009). Leaf material was ground in a Retsch MM 301 ball mill. Silica concentrations were assessed ICP-MS at the Utah State University Analytical Laboratory; this technique also assessed the concentrations of phosphorus and potassium, among other elements. Carbon and nitrogen concentrations were assessed using with a Perkin Elmer 2400 Series II Elemental Analyzer at the Lamont-Doherty Earth Observatory of Columbia University.

## Results

### *Experiment 1: Pairwise preferences*

Ranking plant species by mean quantity of leaf tissue eaten by grasshoppers, the short-statured C4 plant *Cleistogenes squarrosa* was the most preferred food item (Fig. 2.1). The tall, robust grass *Achnatherum sibiricum* was by far the least preferred. Looking more in depth, the relative preference of each species can be assessed in the context of which comparison species was present. More leaf tissue of *Cleistogenes* was eaten than any of its comparison plant species (Fig. 2.2), significantly so for all except *Stipa* or *Agropyron* (Table 2.1). The sedge *Carex* was significantly less preferred than either of the grasses *Cleistogenes* or *Agropyron*, and still more preferred than the grass *Achnatherum*. The previously presumed preferred item, *Leymus chinensis*, was only preferred over *Achnatherum*, and was still less preferred than *Cleistogenes* (Table 2.1, Fig 2.2). Of seven leaf traits examined, only leaf carbon concentration and leaf nitrogen concentration explained significant variation in the feeding preferences. Both greater C and greater N lead to lower feeding preference (Fig. 2.3).

### *Experiment 2: Feeding behavior in the field*

Field observations found that *Oedaleus asiaticus* spends 2.2 min / hr feeding, which did not vary significantly by sex or instar. Feeding bouts lasted an average of 4.5 min, and both feeding and walking activity was strongly dependent on ambient air temperature. Grasshoppers were active nearly 70% of the time when air temperature was between 25-29°C, but only approximately 50% of the time when air temperature was 16-



18°C. Activity tended to decrease with rainfall, despite little variation in precipitation during observations (data not shown).

Across all cages, which varied in plant community composition, *Stipa* was both the most common plant species and the most frequent food item. *Cleistogenes*, the preferred food item in the pairwise preferences experiment, was consumed roughly as often as expected, based on its relative abundance. Accounting for plant community composition, the abundance of each of the six plant species observed to be consumed (*Stipa*, *Leymus*, *Carex*, *Cleistogenes*, *Agropyron*, and *Koeleria*) was a significant factor in determining the amount of that species eaten. Only for two species were the abundances of other species important factors in determining how much time was spent eating it: for *Agropyron*, abundance of *Leymus* increased time spent eating *Agropyron*, while for *Cleistogenes*, abundance of *Stipa* decreased time spent eating *Cleistogenes* (Table 2.2).

### ***Experiment 3: Feeding, clipping and Si addition***

In the pot experiment on the interactive effects of silica addition and herbivory on the dominant rhizomegrass *Leymus* and dominant needlegrass *Stipa*, nearly all of the transplanted individuals of *Stipa* died. Therefore, only results for *Leymus* are shown. Aboveground biomass at harvest was significantly reduced by grasshopper herbivory, but not by the mechanical clipping treatment. Silica addition did not alter the biomass at harvest (Table 2.3). Silica concentration in the leaf tissue of *Leymus* was significantly increased by the combination of grasshopper herbivory and silica addition, but not by either factor alone (Table 2.3, Fig. 2.5). Silica concentrations correlated negatively with C, N, and P on a mass basis. Silica did not vary significantly with maximum

photosynthetic rate or leaf mass per unit area, and tended to positively covary with aboveground biomass at harvest (Fig. 2.6).

Over a survey of fields where sheep stocking rate was experimentally manipulated, high sheep grazing significantly increased silica concentrations in the leaf tissue of *Leymus*, while significantly decreasing it in the leaf tissue of *Stipa* (Fig 2.7).

## Discussion

Results show that the rhizomegrass *Leymus chinensis*, the assumed preferred food item, is in fact not highly preferred, and when the bunchgrass *Stipa grandis* is present the grasshopper *Oedaleus asiaticus* will feed on it. The results from Experiment 1 demonstrate that feeding preferences for *O. asiaticus* do exist within the graminoids, in particular with the inconspicuous, thin-leaved C4 grass *Cleistogenes squarrosa* always being the preferred food item. However, when feeding behavior was directly observed in the field, these preferences are overwhelmed by the stronger tendency of *O. asiaticus* to allocate feeding effort mainly according to the relative abundance of graminoids present, regardless of the preferences observed in the laboratory settings. Focusing on the rhizomegrass *Leymus chinensis*, there does appear to be an increase in silica concentration in response to herbivory by both grasshoppers and sheep, which may explain in part why this dominant and conspicuous grass is less preferred.

In investigating which leaf traits explain the differences in feeding preference, only two traits were significant of the seven examined (leaf C and N concentration, C:N, leaf mass per unit area [LMA], maximum photosynthetic rate, height, leaf:stem ratio). Greater C concentration in leaf tissue lead to significantly less preference, as does greater

N concentration (Fig 2.3). This pattern is largely driven by *Achnatherum sibiricum*, which has high C, high N, and is strongly avoided by *O. asiaticus*. Examining relative preferences after removing *Achnatherum*, relative preferences could not be explained by nutrient concentrations for these plants. C:N ratio was not a significant predictor of relative preferences, surprisingly. Leaf thickness, as measured by leaf mass per unit area, is closely related to measures of leaf toughness (Díaz *et al.* 2001), but also did not affect relative preferences among the graminoids. Among the common plants of the Inner Mongolian grasslands, the LMA of the most preferred food item in the pairwise comparisons, *Cleistogenes squarrosa*, is the lowest of all the graminoids (72 g/m<sup>2</sup>). However, the LMA of the most commonly consumed food item in the field, *Stipa grandis*, is among the highest of all the plants measured (167 g/m<sup>2</sup>), with only an *Iris* and an *Allium* species having thicker leaves (data from Chapter 3). Therefore, factors other than leaf N concentration or this simple measure of leaf thickness drive feeding preferences.

Why is *Achnatherum* so strikingly avoided? The grass does not have dramatically higher C (46.7%) and has the highest N concentrations (1.98%) of the graminoid species investigated here. Other research at the same site has confirmed the strong avoidance of *Achnatherum* despite no clear difference in nutritional content of the grass compared to other graminoids (Zhang *et al.*, in press). Anecdotally, *Achnatherum* is commonly observed in large clumps in grasslands heavily grazed by sheep, even when all other grasses are consumed. This species would appear to be a key candidate for investigation of secondary compounds, such as alkaloids, phenolics, or endophytic fungi which may be

detering both insect and sheep herbivores. N-rich compounds such as alkaloids would be the primary focus.

The density of grasshoppers used in Experiment 2, two individuals per 0.25 m<sup>2</sup> cage, or eight per m<sup>2</sup>, corresponds to a light-to-intermediate density. Heavy herbivory in the middle of the growing season clearly greatly suppresses plant production (Lu *et al.* 2008). Recent work has found that high density of *O. asiaticus* at this site (10-50 individuals per m<sup>2</sup>) diminishes the both the quantity and quality of preferred food plants, by reducing N and P concentrations in remaining leaves. At the same time, that research showed that the nutrient concentrations of non-preferred food plants increased (Zhang *et al.*, in press), highlighting the importance of examining top-down effects of herbivory in a community context. Extrapolating from the work of Zhang *et al.* and from the results presented here, it is possible that greater herbivory by *O. asiaticus* would both increase plant diversity (by preferentially feeding on the dominant plants (as seen in Experiment 2) and also increase the ability of subdominant plants to acquire essential nutrients.

In examining the response of one dominant plant species, *Leymus chinensis*, to both silica addition and herbivory, I found that *Leymus* does appear to increase leaf silica concentrations only when sufficient plant-available silica is available and at the same time when experiencing grasshopper herbivory. This result suggests that *Leymus* may sit on the "defending" side of the growth-defense tradeoff axis, and explain in part why this species is not highly preferred by *O. asiaticus*. However, since the other dominant plant species, *Stipa grandis*, did not survive in this experiment, I am not able to directly compare where that species sits on the growth-defense axis. One piece of evidence, from the survey of *Leymus* and *Stipa* leaf Si concentrations across the sheep stocking rate intensity experiment, shows that *Stipa* in fact had less Si under high herbivory (Fig. 2.7), suggesting that this dominant plant produces Si-poor, quick-growing leaves in response

to herbivory, placing it at the opposite side of the growth-defense tradeoff axis from *Leymus*. Extrapolating these results, it is possible that the extremes of this axis represent the most successful strategies in the Inner Mongolian grasslands. If that is the case, and given that herbivory by *O. asiaticus* in the field was observed principally on whatever species happened to be dominant in a community, herbivory may be maintaining the variation along this growth-defense axis.

The focal grasshopper species, *Oedaleus asiaticus*, is of particular interest both because it is a large and common grasshopper at in Inner Mongolia (Kang & Chen 1992). This species is considered a serious economic pest, and is a graminivorous generalist, which separates its niche from the several forbivorous and omnivorous grasshopper species with which it coexists (Kang & Chen 1994). This species has been hypothesized to be form swarm under high density. However, while the black color morph exhibits substantially greater mass, higher metabolic rates, and is found at higher densities than the green morph, neither morph has significant flying ability, and thus this species is unlikely to be a locust species (Cease et al. 2010).

The results presented here demonstrate that a combination of approaches is necessary for assessing the how the top-down effects of herbivory may play out for plant communities. A controlled experiment demonstrated that the grasshopper *Oedaleus asiaticus* has a strong preference for a thin-leaved, short-statured plant, *Cleistogenes squarrosa*, but the preferences observed in the lab were not detectable in the field. Increase in leaf silica of *Leymus chinensis* and decrease in leaf silica of *Stipa grandis* in response to herbivory, as well as the strong avoidance of the fairly N-rich grass *Achnatherum sibiricum*, demonstrated that antiherbivore defenses may explain feeding

preferences of grasshopper in this grassland system. Extending this work will help to the top-down effects of herbivory on grasslands, and integrate herbivory more fully into research on terrestrial biodiversity-ecosystem functioning relationship.

## Acknowledgments

Arianne Cease, Shuguang Hao, and Guangming Zhang contributed to the experimental designs, grasshopper collection, and to understanding aspects of grasshopper life history. Georgia Hart and Xing Jing provided invaluable assistance in carrying out these experiments. Yongfei Bai generously provided space within the 10-year fenced fields and in the areas surrounding the Inner Mongolian Grassland Ecosystem Research Station to carry out these experiments.

## Tables and Figures

Table 2.1. Feeding preferences of *Oedaleus asiaticus* in a controlled environment.

Values shown are Tukey post-hoc tests for an analysis of variance of the percent of the focal species eaten, with respect to each comparison species; positive values indicate greater preference of the first species in the pair. Significant differences highlighted in bold. Ac = *Achnatherum sibericum*, Ag = *Agropyron cristatum*, Ca = *Carex duriuscula*, Cl = *Cleistogenes squarrosa*, L = *Leymus*, S = *Stipa grandis*.

Pair	Difference	Lower bound	Upper bound	<i>P</i>
Ag-Ac	78.73	53.42	104.04	<b>&lt;0.001</b>
Ca-Ac	52.27	26.12	78.42	<b>&lt;0.001</b>

Ca-Ag	-26.46	-51.95	-0.96	<b>0.037</b>
Cl-Ac	83.35	57.39	109.32	<b>&lt;0.001</b>
Cl-Ag	4.63	-20.68	29.94	0.995
Cl-Ca	31.08	4.93	57.23	<b>0.010</b>
L-Ac	53.95	27.98	79.92	<b>&lt;0.001</b>
L-Ag	-24.77	-50.08	0.53	0.059
L-Ca	1.68	-24.47	27.83	1.000
L-Cl	-29.40	-55.37	-3.44	<b>0.016</b>
S-Ac	59.46	33.31	85.62	<b>&lt;0.001</b>
S-Ag	-19.26	-44.76	6.24	0.254
S-Ca	7.19	-19.14	33.53	0.970
S-Cl	-23.89	-50.04	2.26	0.095
S-L	5.51	-20.64	31.66	0.990

Table 2.2. Summary of general linear model of observed time spent eating one focal species, *Cleistogenes squarrosa*, by the grasshopper *Oedaleus asiaticus*, with respect to the composition of dominant plant species.

Factor	Estimate	SE	<i>z</i>	<i>p</i>
Intercept	-1.97	1.11	-1.78	0.075
<i>Stipa</i>	-2.99	1.52	-1.97	<b>0.049</b>
<i>Leymus</i>	-0.35	2.03	-0.17	0.862
<i>Agropyron</i>	0.96	1.44	0.67	0.504
<i>Cleistogenes</i>	10.93	4.92	2.22	<b>0.026</b>
<i>Carex</i>	-8.97	5.26	-1.71	0.088

Table 2.3. Summary of general linear model of *Leymus chinensis* to experimental silica addition, crossed with mechanical wounding or grasshopper herbivory in semi-natural conditions. Response variables are aboveground biomass at harvest after 57 days and silica concentration in leaf tissue. Estimates show change with respect to the control.

<i>Response</i>	<i>Treatment</i>	<i>Estimate</i>	<i>SE</i>	<i>t</i>	<i>P</i>
Biomass	Control	7.26	0.87	8.36	<0.001
	+Si	0.84	1.33	0.64	0.527
	Clipping	-0.01	1.23	-0.01	0.994
	Clipping+Si	-0.17	1.17	-0.14	0.886
	Herbivory	-4.16	1.23	-3.38	0.001
	Herbivory+Si	-5.57	1.23	-4.54	<0.001
Si	Control	2075.25	82.22	25.24	<0.001
	+Si	62.03	125.59	0.49	0.623
	Clipping	15.58	116.27	0.13	0.894
	Clipping+Si	14.39	112.04	0.13	0.898
	Herbivory	175.35	121.95	1.44	0.156
	Herbivory+Si	321.61	135.45	2.37	0.021



Figure 2.1 Quantity of leaf tissue eaten by *O. asiaticus* in a controlled environment.

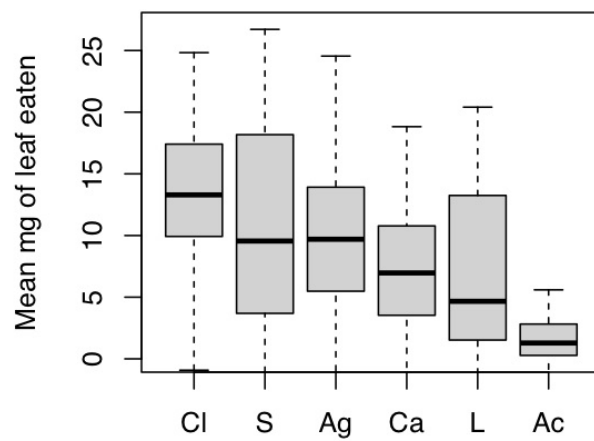


Figure 2.2 Relative consumption preference of common plants of Inner Mongolia by *O. asiaticus*.

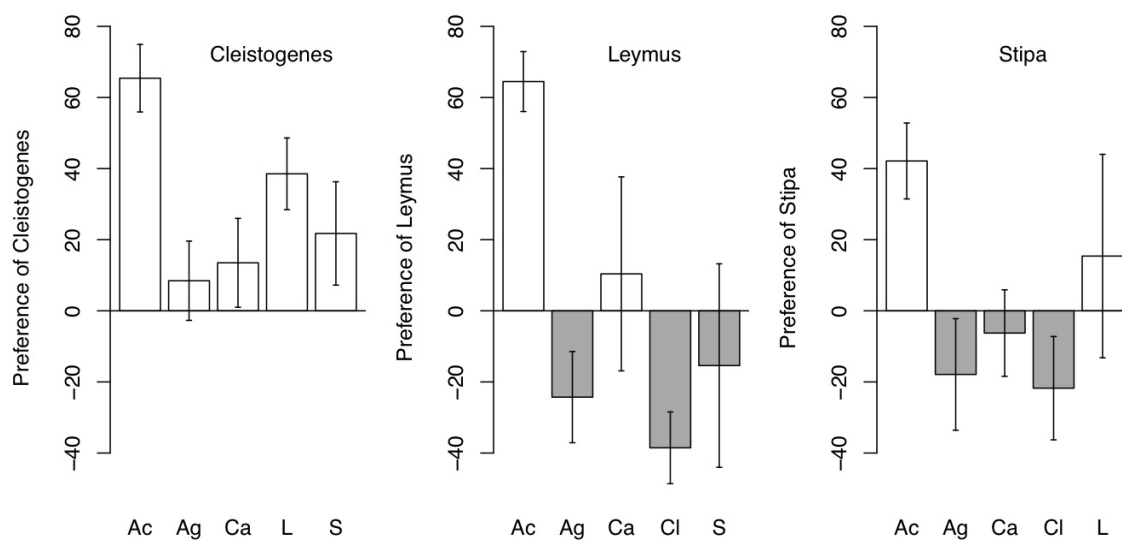


Fig. 2.3. Relative difference in consumption preference for each of six plant species by *O. asiaticus* compared to difference in carbon and nitrogen concentrations in leaf tissue. Sign of difference values in consumption preference is arbitrary for each species pair, but values are aligned with the pair in the same order for differences in element concentrations.

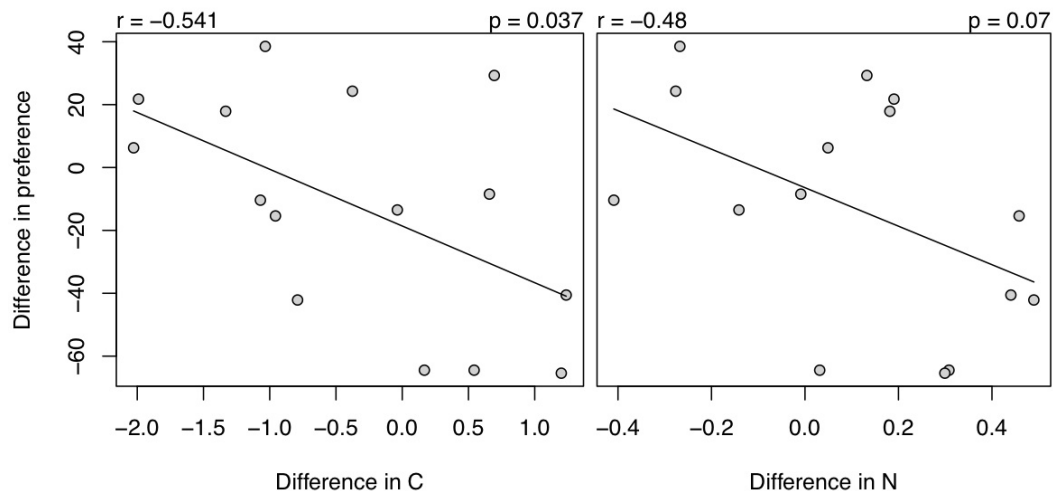


Figure 2.4. Observed feeding behavior of *O. asiaticus* in the field.

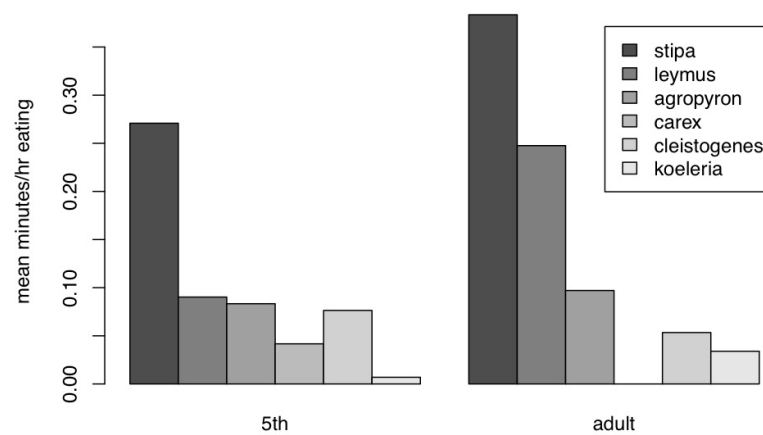


Figure 2.5. Silica concentrations in leaf tissue of *Leymus chinensis* under experimental Si addition crossed with mechanical clipping and grasshopper feeding.

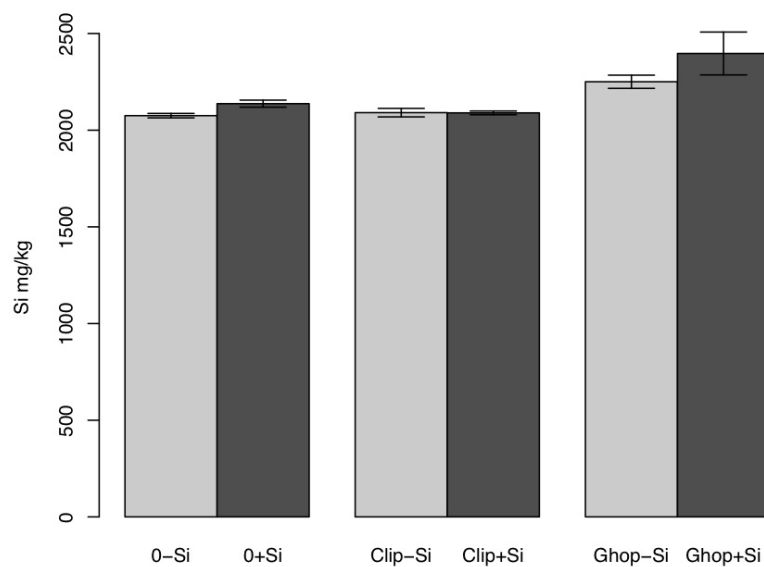


Fig. 2.6. Correlation between leaf traits, as well as aboveground biomass, under experimental Si addition.

Leaf elements and biomass log-transformed.  $A_{\max}$  = Maximum photosynthetic rate.

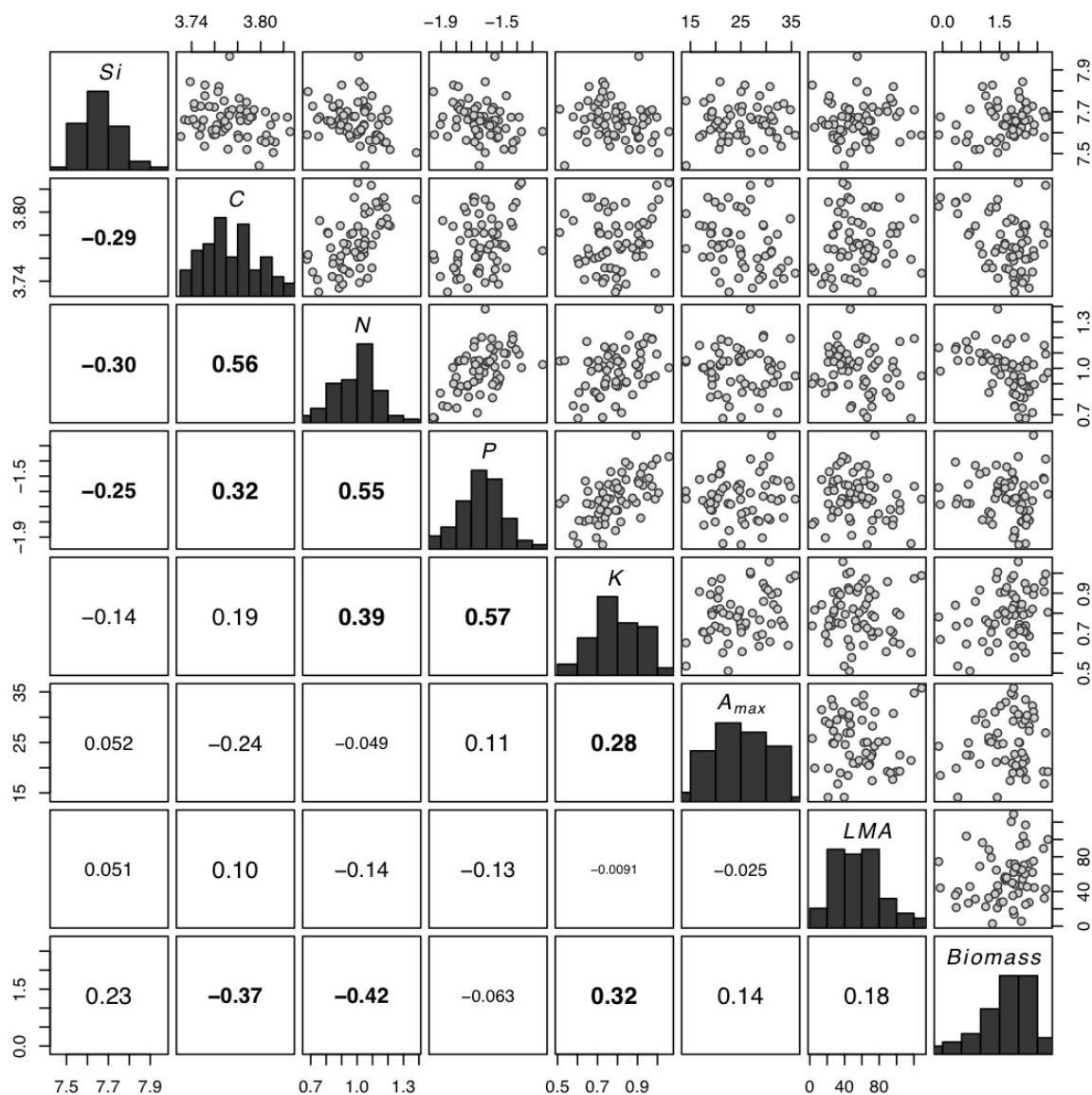
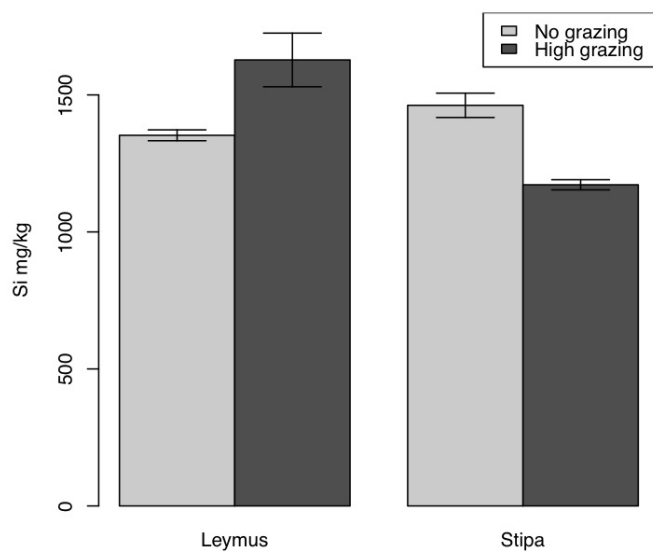


Fig. 2.7. Silica content of the two dominant grasses, the rhizomegrass *Leymus chinensis* and the bunchgrass *Stipa grandis* in response to sheep stocking rate.



### **CHAPTER 3. HIGH NICHE OVERLAP IN GRASSLAND PLANT COMMUNITIES IRRESPECTIVE OF HERBIVORY IN INNER MONGOLIA, CHINA**

#### **Summary**

The relative importance of limiting similarity versus habitat filtering in community assembly has been a focal point in research on species coexistence, with new approaches recently developed to take advantage of functional trait data. Because herbivory in grasslands strongly influences plant species community composition and structure, elucidating the role of herbivores in assembly processes has the potential to resolve how biodiversity is maintained in such communities where resource use and other axes of life history variation may be difficult to capture.

I examined plant community assembly under manipulations of grasshopper or sheep herbivory using an experimental, trait-based approach in the grasslands of Inner Mongolia, China. I investigated changes in plant community composition and structure under short-term grasshopper grazing and long-term sheep grazing at different intensities, and directly measured of niche overlap based on plant functional traits using a novel niche overlap index based on convex hull volume. Intersections of hull volumes for species, weighted by abundance, were made based on extensive ecophysiological and morphological trait measurements.

Niche overlap was significantly larger than expected under a null model in all treatments of both experiments, indicating strong environmental filtering with no evidence for limiting similarity. No effect of grasshopper herbivory at the neighborhood

scale or sheep stocking rate at landscape scale was detected on niche overlap. However, niche overlap fell both within the growing season in the grasshopper experiment and in the four years between census periods in the sheep grazing experiment. Environmental filtering was the dominant processes influencing assembly in these communities. There is no evidence for herbivory acting to strengthen environmental filtering. The surprisingly strong signal of environmental filtering, irrespective of herbivory, reflects the high functional similarity of these communities.

## Introduction

Identifying the processes influencing the assembly of species into communities has long been a major goal of community ecology. In particular, understanding the relative importance of environmental filtering and limiting similarity in governing community assembly remains a major challenge. Environmental filtering refers to exclusion of those species from the regional species pool that lack traits necessary to persist in the local environment. Limiting similarity refers to interspecific competition eliminating species whose resource use traits are too similar. While the relative balance between these two factors in determining which species coexist has been explored for a long time (MacArthur & Levins 1967), recent efforts have focused on the use of functional traits, particularly in plants, to detect patterns of clumping or dispersion in traits as indicators of the ecological factors shaping communities (Weiher *et al.* 1998; Grime 2006).

The influence of herbivory on the balance between the processes of environmental filtering and limiting similarity has not been addressed, despite the clear

importance of herbivory in influencing the composition and structure of plant communities (Huntly 1991; Carson & Root 2000). If generalist herbivores in these communities act to remove dominant species, and if dominant species are similar in trait values, the resulting communities may be composed of subdominants with a greater range and less overlap in functional traits. Thus generalist herbivores could act to reduce the signal of environmental filtering. In contrast, if generalist herbivores act as an additional "filter" by removing palatable and nutritious plants first, the resulting communities may be composed of species all with similar traits. Thus in this case, generalist herbivory could act to enhance the signal of environmental filtering.

To a large extent, the challenge in addressing the question of community assembly stems from the large number of factors that influence community composition and structure, and the difficulty of identifying which factors are dominant among them. Thus, tackling the question of community assembly in a natural setting is ideally done with relatively simple system. The Inner Mongolian grasslands provide one such case: in this system, strong environmental factors, namely a short growing season with limited precipitation, interact with strong biotic factors, namely competition among a limited number of plant species for light and space. In addition, these grasslands are managed for sheep grazing and are also under pressure from grasshopper herbivory.

Developing and employing measures of how similar species are with respect to functional traits has become the predominant method for addressing the relative importance of limiting similarity and environmental filtering in structuring communities (Weiher & Keddy 1995). In plant communities, this approach has demonstrated that both limiting similarity and habitat filtering can act to structure plant communities, depending



on the spatial scale examined (Kraft et al. 2008). Nevertheless, other studies based on fewer traits have found no signal of either process in structuring old field plant communities (Schamp et al. 2007). In this study I propose a measure of similarity based on convex hulls (see below) that takes into account trait correlations at the individual level and the abundance of species within communities.

This study asks two questions: 1. what is the relative importance of limiting similarity and environmental filtering in structuring plant communities in the Inner Mongolian grassland? and 2. to what extent does herbivory alter the importance of these processes in structuring plant communities? This study also presents a novel metric of community similarity, based on convex hull volume (D. Bunker, D. Flynn, S. Naeem, in prep.; Cornwell et al. 2006), which directly measures the overlap in functional traits related to resource acquisition, taking into account intraspecific trait variability and abundance at the plot scale. I take advantage of two experiments testing the impact of herbivores on plant community structure. The two experiments differ in spatial and temporal scale, as well as identity of the herbivores.

## **Materials and methods**

### ***Study Site***

The study was carried out near the Inner Mongolia Grassland Research Station (43°38'N, 116°42'E) of the Institute of Botany in the Chinese Academy of Sciences. Located in the Xilin River catchment, this area has a continental, semi-arid climate, with mean annual precipitation of 334 mm and mean annual temperature of 0.7°C. The typical

steppe ecosystem is dominated by C3 grasses, particularly the perennial rhizome grass *Leymus chinensis* and the perennial bunchgrass *Stipa grandis* (Bai et al. 2004). Given the relatively simple plant community structure, with fewer than 20 common plant species, this community well-suited for examining how functional traits reflect the processes of habitat filtering or limiting similarity in structuring communities. This system is strongly precipitation-limited, with aboveground net primary productivity rising sharply with precipitation even in sites with widely divergent species composition (Bai *et al.* 2004; Bai *et al.* 2008). In addition, there is evidence that species richness covaries across spatial scales with productivity in this region (Bai et al. 2007). Biodiversity-ecosystem functioning research in these grasslands has revealed that temporal complementarity in plant populations drives a significant biodiversity-stability relationship (Bai et al. 2004), highlighting the role of temporal niche separation in explaining maximum abundances of the dominant functional groups. The Inner Mongolian grasslands thus provide a test system to disentangle the relative importance of environmental filtering and limiting similarity in community assembly, and how herbivory alters the balance of these forces at different spatial and temporal scales.

### ***Plant Communities***

I took advantage of two existing experiments evaluating the effects of herbivores on plant communities in Inner Mongolia, China. This study focuses solely on the aspects of niche overlap resulting from these herbivory treatments, not on the overall patterns of plant community structure or responses of particular species to the herbivory treatments, which are presented elsewhere (Flynn et al. in prep, Wang et al. in prep).

*Grasshopper herbivory experiment, neighborhood-scale.* In the first experiment, 200 0.25 m<sup>2</sup> plant neighborhoods located in several blocks in an experimental field fenced for 10 years to exclude sheep grazing were surveyed in early June 2009. Here, I use the term "neighborhood" because the scale of the plots was small enough to assume that individuals would likely interact. Individuals were counted and relative cover estimated for all species, and average height per species was measured. Following the survey, on June 22 2009 neighborhoods were enclosed in 0.25 m<sup>2</sup> diameter x 1 m nylon mesh cages to exclude grasshoppers and other insect herbivores. The blocks of plots were arrayed over a wide range of plant community compositions, resulting in communities with richnesses of 3 - 10 species and a range of compositions, including communities dominated variously by the bunchgrass *Stipa grandis* and the rhizomegrass *Leymus chinensis* (Table 1).

The grasshopper manipulation involved the addition of two individuals of the generalist *Oedaleus asiaticus*, one male and one female 3<sup>rd</sup> instar, to a subset of the cages (see below). For this species, sexing even early instars is straightforward, as ovipositors are distinct by the 3<sup>rd</sup> instar. *O. asiaticus* is a large and common grasshopper at in Inner Mongolia, typically peaking in density in mid-July (Kang & Chen 1992). This species is considered a serious economic pest, and is a graminivorous generalist, which separates its niche from the several forbivorous and omnivorous grasshopper species with which it coexists (Kang & Chen 1994). All individuals used in the experiment were sweep-netted from a heavily-grazed field site approximately 5 km from the experimental site. There were four treatments, with 50 cages each: black grasshopper (the black color morph of *O. asiaticus*, which is the larger, "gregarious" form), green grasshopper (the smaller,

"solitary" form), clipped, and controls. Thus a total of 200 grasshoppers was used in the study, 100 of each color morph. Treatments were assigned to rows of cages within blocks in a regular pattern. For the purpose of this study, the two color morphs are assumed to represent different intensities of grasshopper grazing, as the black morph has not only larger body size (femur length, abdomen length, and weight), but also a higher metabolic rate (Cease et al. 2010). Experimental clipping was performed immediately after caging, in which all plant material was cut to ground level and left within the cages. The clipping treatment mimicked severe herbivory by removing all aboveground biomass, and was additionally designed to measure the regrowth ability of these plant species for a parallel study (Flynn et al., in prep.).

All cages were harvested to the ground on July 28 2009. Aboveground biomass was sorted to species, separated into leaf and stem material, and oven dried at 60°C for at least 24 hr before being weighed. Grasshoppers were collected, weighed, and dried for future analysis. Leaf material from grasshopper experimental cages was further separated into leaf tissue with obvious chewing damage and undamaged tissue.

*Sheep stocking rate experiment, landscape scale.* Since 2005, a Sino-German collaboration has been experimentally manipulating sheep grazing intensity at the field scale near the IMGERS field site. This experiment, "Matter Fluxes in Grasslands of Inner Mongolia as Influenced by Stocking Rate" (MAGIM, <http://magim.net>), is aimed at elucidating how rangeland management affects grassland biotic and abiotic processes. For the purpose of this study, plant community composition data from the first full year of the experiment, 2005, and from 2009 were used from replicate fields with 0, 1.5, 3,

4.5, 6, 7.5, and 9 sheep per hectare. In 2005 plant community composition data were pooled from the three replicate fields per treatment, while plot-level data were available for 2009, resulting in a total of 76 plots. Full descriptions of the experiment, including details on how sheep densities were maintained in fields, can be found in Schönbach et al. (2009).

### ***Plant Trait Measurements***

I collected trait data for 370 individuals, spanning 18 species and six families, across the growing seasons of 2008 and 2009 (Table 1). In 2008 and 2009, traits measured were leaf mass per unit area (LMA), height, maximum photosynthetic rate ( $A_{\max}$ ), water use efficiency, C and N concentrations, length of longest leaf, and biomass of leaves and stems (Table 2). Standard methods were used for the measurement of each of these traits (Cornelissen et al. 2003). Plants were selected from communities adjacent to the grasshopper study cages of each block, and sampled throughout the growing season. Measurements were made on a minimum of five individuals of every species observed in the grasshopper study cages. All measurements were conducted on an individual basis, where an individual constituted one ramet for rhizomatous plants and one bunch for bunchgrasses. In the field,  $A_{\max}$  was measured on a selected leaf or small group of leaves for thin-leaved grasses, using a Li-Cor 6400 Portable Photosynthesis System (Lincoln, Nebraska) with a block temperature of 25°C and CO<sub>2</sub> of 400 ppm; five measures were made after carbon exchange stabilized. Plant height and length of longest leaf were measured in the field, and then each individual was placed in a paper envelope, inside a plastic bag to maintain moisture content. Leaf area was scanned immediately

upon return from the field, and all aboveground material was dried at 60°C for 24 hr prior to weighing. Leaf material was ground in a Retsch MM 301 ball mill and analyzed for C and N with a Perkin Elmer 2400 Series II Elemental Analyzer at the Lamont-Doherty Earth Observatory of Columbia University.

Research using functional traits include those investigating community assembly processes (Ackerly & Cornwell 2007; Kraft *et al.* 2008; Cornwell & Ackerly 2009), community responses to land use change (Flynn *et al.* 2009; Laliberté *et al.* 2009), or potential for communities to affect ecosystem properties (Chapter 4; Clark *et al.*, in review; Griffin *et al.* 2009). In all of these studies, the traits selected and the number of traits used critically influence the outcome of the analysis. The set of nine traits used in this study (Table 2) were chosen to reflect above-ground resource acquisition and general life history strategies. If other trait data had been available, such as root:shoot ratio or seed size, the results may have demonstrated different magnitudes of niche overlap, by reflecting other aspects of the niche, in particular belowground processes, which are not addressed by the current study. Leaf mean area, photosynthetic rate, and leaf nitrogen concentration in particular tend to covary along a "leaf economics spectrum", reflecting variation from fast carbon gain and leaf turnover to slow carbon gain and longer leaf life span (Wright *et al.* 2004; Poorter *et al.* 2009). Being tied closely with plant life history strategies, these traits represent good candidates for examining the balance of limiting similarity and environmental filtering in shaping plant communities.

### ***Measure of Niche Overlap***

Many measures of habitat filtering and limiting similarity have been developed recently based on trait overlap (Stubbs & Wilson 2004; Mouillot *et al.* 2005; Cornwell *et al.* 2006). An ideal measure of niche overlap based on traits would be multivariate, taking into account abundance of species in communities and their intraspecific variation. Here I present a new metric of niche overlap, based on the concept of convex hull volume.

Convex hulls describe the minimum volume required to contain a set of points in multivariate space and represent the multivariate range of a set of data. Applying this technique from computational geometry to species traits allows the calculation of the "volume of trait space occupied by species in a community" (Cornwell *et al.* 2006). In ecology, this method has been applied to measures of plant trait diversity in oldfields (Schamp *et al.* 2007) and in response to disturbance (Pausas & Verdu 2008). Convex hull volume has been shown to have a higher degree of sensitivity for discriminating among community assembly processes than all other proposed measures using artificial data sets (Mouchet *et al.* 2010).

Our extension of convex hull volume differs from previously described methods by 1. accounting for intraspecific variation using individual-level trait data, 2. accounting for species abundances, and 3. being able to calculate the niche overlap of species in a community as the amount of hull volume intersection between species. This assumes that the traits chosen reflect the entirety of the relevant aspects of the niche. While previous studies of functional trait diversity have been almost exclusively based on mean values by species (but see Cianciaruso *et al.* 2009), here I use trait values at the individual level, without aggregating to species mean, to more fully capture the intraspecific variation in

plant functional traits. In this formulation, hull volumes are calculated for species within a community, and with individuals as the vertices of species hulls. To account for abundance, I weight the trait values of species present in a given community by the log of their abundance (count data on stems for plant species for grasshopper experiment, dry aboveground biomass for sheep experiment). Thus, for a given species, a hull volume will be calculated separately for each community, based on the same set of trait values for that species but a unique value for the abundance. While this abundance-weighting procedure has been shown to result in an index that has low power for predicting the biodiversity effect in an experimental biodiversity-ecosystem function study (C. Clark, D. Flynn, P. Reich, and B. Butterfield, in review), its utility in evaluating community assembly processes has not been evaluated previously. The abundance-weighted convex hull volume incorporates the aspects of functional richness, functional evenness, and functional divergence as defined by Mason et al. (2005). The net species volume reflects the total amount of functional space occupied by the assemblage (i.e., functional richness, or the multivariate range in trait values of a community), while the relative volume reflects the evenness of the distribution of species in functional space and the niche overlap is related to the amount of functional divergence in the community.

To distinguish between expected niche overlap based on the number of species present and the observed values, I ran null models in which a random draw of species from the pool of species present in each experiment was performed 1001 times at each species richness level, and at each census time period (i.e., initial grasshopper communities, grasshopper communities at harvest, 2005 sheep communities, and 2009 sheep communities). Observed overlap values for each plot were compared to those of



null communities with the same number of species with trait data available (i.e., realized species richness given the trait data set of this study). Abundances were assigned from random uniform draws with replacement of the observed abundances within each experiment at each time step. Significant deviations from expectation were determined at  $\alpha = 0.05$ . All calculations, null models, and analyses were performed in R v2.11.1 (R Development Core Team 2010). Convex hull intersections were performed using the program *polymake* (Gawrilow & Joswig 2000).

## Results

Significant correlations at the individual level were observed for many pairs of traits (Appendix S1). In particular, plant size traits (height, length of longest leaf, and aboveground biomass) correlated positively with leaf C concentration ( $r = 0.38, 0.60$ , and  $0.29$ , respectively, for log-transformed values). LMA, leaf N concentration, and maximum photosynthetic capacity on an area basis ( $A_{\text{area}}$ ), the three traits composing the "leaf economics spectrum" (Wright et al. 2004), were not all tightly related, with LMA not significantly correlated to either N or  $A_{\text{max}}$ . However, given the overall degree of correlation between traits, principal components analysis was successful at reducing the number of axes, with the first three principal components accounting for 64% of the variation in the 9 traits used (Appendix S2). The first principal component reflects metabolic rate ( $A_{\text{max}}$ , N, and conductance) versus plant size, while the second reflects leaf structure (C and LMA), and the third reflects plant size (area, aboveground mass, LMA, and longest leaf).

Observed niche overlap measures for all plant communities far exceeded the expected values from null communities. That is, observed niche overlap values from plant communities, regardless of which census period or under which herbivore treatment, were much higher than would be expected if the community represented a neutrally assembled community. Greater overlap values were observed after 1 month of grasshopper herbivory in the neighborhood experiment (Fig. 2A) at all species richness levels. Grasshopper treatments, including feeding by the gregarious, larger black morph of *O. asiaticus* and the solitary, green morph of *O. asiaticus*, as well as mechanical clipping, resulted in no significant difference in niche overlap (Table 4).

For plant communities under sheep grazing, observed niche overlap values and total convex hull volumes also far exceeded null expectations at all species richness levels (Fig. 2C-D). Across all sheep stocking treatments, both species richness and niche overlap declined in the five years between census periods. While the change in niche overlap was the least for the control plots, no significant effect of the stocking rate treatments was detected for the niche overlap measures (Table 4).

## Discussion

Irrespective of identity, intensity, or time scale of herbivory, nearly all plant communities exhibited much greater overlap than predicted by the null model. Two aspects of this result are surprising. First, the degree of niche overlap, as measured by overlap in hull volumes of species in trait space, exceeded the null expectation in all cases. Second, neither grasshopper herbivory by either color morph over one growing season nor sheep herbivory across a range of stocking rates altered the composition or high degree of niche

overlap in the plant communities. Thus, generalist herbivory neither enhanced nor reduced the strong signal of environmental filtering in the assembly of these communities.

In addition to the choice of traits, the choice of niche overlap metric can also have important implications. Functional diversity indices have been developed to address two issues in community ecology: predicting changes in ecosystem function (largely focused on aboveground biomass in grasslands), and detecting the imprint of different community assembly processes. Convex hull volume and its abundance-weighted variation have been shown to be surprisingly poor predictors of grassland aboveground biomass in a multi-index comparison (Clark et al., *in review*), yet the same index emerged as the most powerful in detecting processes of community assembly (Mouchet et al. 2010). Indices based on the multivariate range of traits, like convex hull volume, may be more appropriate for addressing community assembly than predicting changes in ecosystem functioning. In all cases, understanding the advantages and limitations of each index of functional diversity is crucial (Petchey et al. 2009).

Calculating niche overlap has a long history in ecology, but recent advances in compilation of functional traits and in computing power together have allowed for a range of new approaches to this issue. In the context of this study, I am most interested in resource niche overlap, as represented by functional traits. As the functional traits chosen here are assumed to reflect the ability of grassland plants to efficiently compete for light, water, and nutrients, the degree of similarity in net resource use, accounting for all of these factors simultaneously. Mouchet and colleagues (2010) reviewed the currently popular functional diversity metrics in part for their utility in discriminating between

different community assembly processes leading to clumped or overdispersed trait patterns, which have long been assumed to correspond to the dominance of limiting similarity or environmental filtering (e.g., Weiher *et al.* 1998; Rabosky *et al.* 2007). Their finding that convex hull volume has high power to discriminate between contrasting community assembly processes gives support for the current study.

Our questions concern how specific niche dimensions can reveal the relative roles of environmental filtering and limiting similarity in species persistence rather than questions about phylogenetic similarity and environmental filtering and limiting similarity. In contrast to the present study, phylogenetic methods frequently been used to assess community assembly processes, often finding evidence for phylogenetic overdispersion, which has been interpreted as evidence of limiting similarity. Such studies have generally found that at small scales communities are more overdispersed than expected, consistent with limiting similarity at the neighborhood level, but that communities are more clustered than expected at larger scales, consistent with environmental filtering dominating at the landscape level (Cavender-Bares *et al.* 2006; Swenson *et al.* 2007; Cadotte *et al.* 2009b). Here, however, I focus on functional traits, rather than phylogenetic similarity, to directly assess whether these functional traits are the axes of variation important to community assembly. Functional traits have the potential to provide insights into specific dimensions of a species' niche, whereas phylogenetic measures reflect a broader range of niche dimensions.

Direct effects of herbivory on plant functional traits were not assessed here, since the trait sampling was destructive and was thus carried out in undisturbed plots surrounding the experimental plots. Previous work has shown increases in specific leaf

area (SLA, the inverse of LMA) in these sites for both *Leymus* and *Stipa* after two years of defoliation (Schiborra et al. 2009). In addition, grazing by *O. asiaticus* at this same study site has been shown to eliminate low-N, high C:N host plants, leaving behind high-N, low C:N plants (Zhang et al., in review). It is possible that variation in the degree of response to herbivory could modify the degree of realized niche overlap, for example by leading to differences in investment in defense versus growth. However, the limited change in trait composition of communities under either grasshopper or sheep herbivory suggests that such direct effects of herbivores on trait phenotypes may not overcome the high similarity in trait composition, but future work would be needed to address the direct effects of herbivory on plant traits related to resource niche overlap.

The high overlap in the resource niche, as reflected by the convex hull volume metric used in this study, demonstrates that limiting similarity does not account for the coexistence of the grassland plant species investigated here. Temporal separation in the timing of peak biomass has previously been shown to be a major mechanism promoting coexistence of the dominant plant species in this study region (Bai et al. 2004). The results presented here highlight the potential importance of temporal niche separation, given the high resource niche overlap. A similar study found no evidence for limiting similarity in plant communities undergoing low-intensity disturbance (Thompson et al. 2010). Intriguingly, grassland plant species with high stoichiometric homeostasis (i.e., limited variation in tissue nutrient ratios despite variation in soil nutrient availability) have been shown to be the more dominant species in this region of Inner Mongolia (Yu et al. 2010). It is possible that plant species coexistence in this region is determined more by the ability for multiple species to maintain steady tissue nutrient ratios despite variation

in nutrient availability, rather than segregation of resources in a niche complementarity perspective.

Niche complementarity, which is based in part on the idea that species have a minimum degree of differentiation in functional traits, has been taken to be the key process underpinning positive biodiversity-ecosystem functioning relationships (Hooper et al. 2005). Thus, our results present a conundrum: given the evidence that there is low degree of separation in niche space at the neighborhood scale, why is niche complementarity such a powerful explanatory factor in biodiversity-ecosystem functioning relationships? Further work should address the question of identifying which traits most accurately reflect the effects of species on ecosystem functioning, with increased attention to belowground traits, as well as which traits most accurately reflect the response of species to the forces of environmental filtering and limiting similarity.

## **Acknowledgements**

Many thanks to YF Bai, F Taube, HW Wang, and P Schönbach for access to the MAGIM sheep grazing experiment data. Thanks to G Hart and X Jing for field assistance and to D Bunker for convex hull code. This research was supported by a National Science Foundation Graduate Research Fellowship to DFBF and National Science Foundation grant to J Wu, J Elser, and S Naeem.

## Tables and Figures

Table 3.1. Summary of grassland plant species sampled for functional traits.

Number: Total number of individuals sampled across years. RA: Relative abundances, based on biomass, across all treatments and sampling period for both plant community data sets, under grasshopper and sheep grazing.

Family	Species	Number	RA - Grasshopper	RA - Sheep
Poaceae	<i>Stipa grandis</i>	40	42.42	41.37
Poaceae	<i>Leymus chinensis</i>	42	19.61	35.49
Poaceae	<i>Agropyron cristatum</i>	24	17.52	6.26
Cyperaceae	<i>Carex duriuscula</i>	35	7.26	7.69
Poaceae	<i>Koeleria cristata</i>	14	3.63	0.59
Poaceae	<i>Cleistogenes squarrosa</i>	35	3.12	4.28
Poaceae	<i>Achnatherum sibiricum</i>	35	3.10	3.00
Liliaceae	<i>Allium tenuissimum</i>	33	2.43	0.09
Poaceae	<i>Poa subfastigiata</i>	18	0.34	0.25
Liliaceae	<i>Anemarrhena asphodeloides</i>	8	0.16	0.02
Iridaceae	<i>Iris tenuifolia</i>	5	0.08	0.03
Liliaceae	<i>Allium condenstatum</i>	12	0.07	0.01
Liliaceae	<i>Allium senescens</i>	5	0.06	0.04
Brassicaceae	<i>Dontostemon micranthus</i>	13	0.05	<0.01
Ranunculaceae	<i>Thalictrum petaloideum</i>	13	<0.01	0.06
Chenopodiaceae	<i>Salsola collina</i>	10	<0.01	<0.01
Chenopodiaceae	<i>Axyris amaranthoides</i>	11	<0.01	<0.01
Chenopodiaceae	<i>Chenopodium glaucum</i>	17	<0.01	<0.01
Totals		370	99.83	99.18

Table 3.2. Summary of trait data. LMA: Leaf mass per unit area.

Trait	N	Mean	SD	Range
Height (cm)	348	28.85	12.56	3.7-69.1
Longest Leaf (cm)	348	16.23	10.36	1.03-40.8
Aboveground Weight (g DW)	332	0.34	0.51	0.01-6.39
Area (cm <sup>2</sup> )	343	1.30	0.63	0.3-5.29
LMA (g m <sup>-2</sup> )	320	102.49	58.48	17.6-437.6
C%	188	44.50	3.42	34.2-53.3
N%	188	2.09	0.71	0.83-5.18
A <sub>max</sub> (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	312	10.46	9.25	0-71.67
Conductance (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	312	0.08	0.07	0-0.55

Table 3.3. Summary of community data. Average species richness shown for each herbivory treatment, by experiment and time period. Number of plots are shown in parentheses.

Communities	Treatments	Time periods	
		Initial - June	Harvest - July
Grasshopper	Control	5.52 (50)	5.78 (50)
	Black	5.30 (50)	5.52 (50)
	Green	5.76 (50)	5.94 (50)
	Clip	5.62 (50)	5.76 (50)
Sheep	Sheep/ha	2005	2009
	0	10.0 (2)	7.1 (8)
	1.5	15.5 (2)	6.0 (14)
	4	14.0 (2)	5.1 (8)
	4.5	13.5 (2)	5.4 (8)
	6	16.5 (2)	5.6 (8)
	7.5	15.0 (2)	5.5 (8)
	9	14.0 (2)	5.6 (8)



Table 3.4. Summary of mixed-effects models for niche overlap. No effect of either grasshopper herbivory at the neighborhood scale or sheep herbivory at the landscape scale was detectable. In both cases, niche overlap values changed substantially between the census periods.

Experiment	Treatment	Estimate	SE	<i>df</i>	<i>t</i>	<i>P</i>
Grasshopper herbivory	Control	2.36	0.16	199	14.69	<0.001
	Black	-0.01	0.22	196	-0.03	0.975
	Green	-0.19	0.22	196	-0.90	0.371
	Clip	-0.27	0.22	196	-1.28	0.204
	Time	0.99	0.10	199	9.73	<0.001
Sheep grazing	Control	516.61	183.53	55	2.81	0.007
	1.5	0.04	0.68	55	0.06	0.955
	3	-0.73	0.76	55	-0.96	0.344
	4.5	-1.14	0.76	55	-1.50	0.140
	6	0.11	0.76	55	0.14	0.888
	7.5	-0.33	0.76	55	-0.44	0.664
	9	-0.48	0.76	55	-0.63	0.533
	Year	-0.26	0.09	13	-2.80	0.015

### ***Figure Legends***

Figure 3.1. Conceptual diagram demonstrating the two modifications to the convex hull volume method introduced in this study. Upper panels show either substantial (A) or no (B) niche overlap, as measured by the intersection in convex hull volumes of two species. In both cases the total volume (CHV) is similar. Niche axes could be trait values or as in this study, principal components from multiple trait values. Lower panels show the same hypothetical communities, but with hull volumes for species adjusted by relative abundances. Species 1 and 3 are dominant and thus hull volumes are expanded, while species 2 and 4 are minor components of the community, and thus have reduced hull volumes.

Figure 3.2. Niche overlap (A-C) and total convex hull volume (B-D) of plant neighborhoods under short-term grasshopper herbivory (top panels) and long-term sheep grazing (bottom panels) by species richness. For both experiments, both observed niche overlap values and total convex hull volumes far exceeded null expectations at all species richness levels. For the plant communities under grasshopper herbivory, across treatments significantly greater overlap was observed at harvest. For plant communities under sheep grazing, across treatments both species richness and niche overlap fell in the five years between census periods.

Figure 3.3. Niche overlap of plant communities under short-term grasshopper herbivory (top panel) and long-term sheep grazing (bottom panel) by experimental treatment. For both experiments, niche overlap was not significantly altered by herbivory or grazing

intensity experiments. Over the course of the growing season, niche overlap increased in the communities under grasshopper herbivory, while over the course of several years, both species richness and niche overlap decreased in the communities under sheep grazing.

Figure 3.1. Conceptual diagram of the abundance-weighted convex hull.

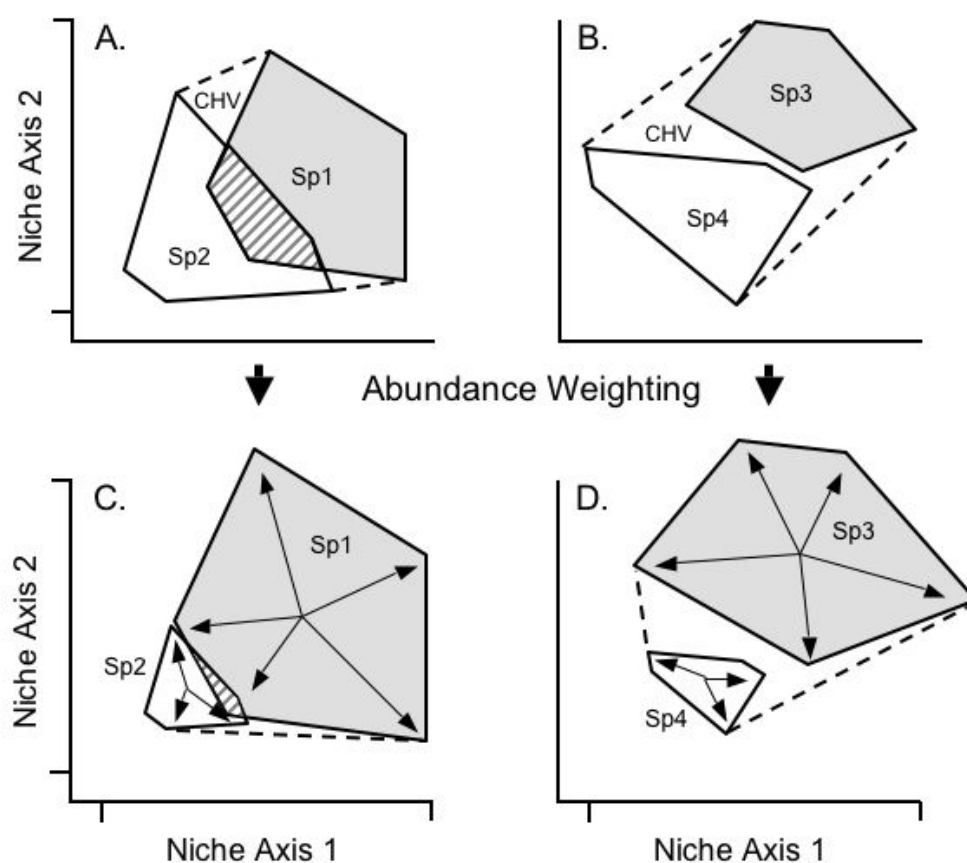


Figure 3.2. Niche overlap and functional richness of plant communities under herbivory.

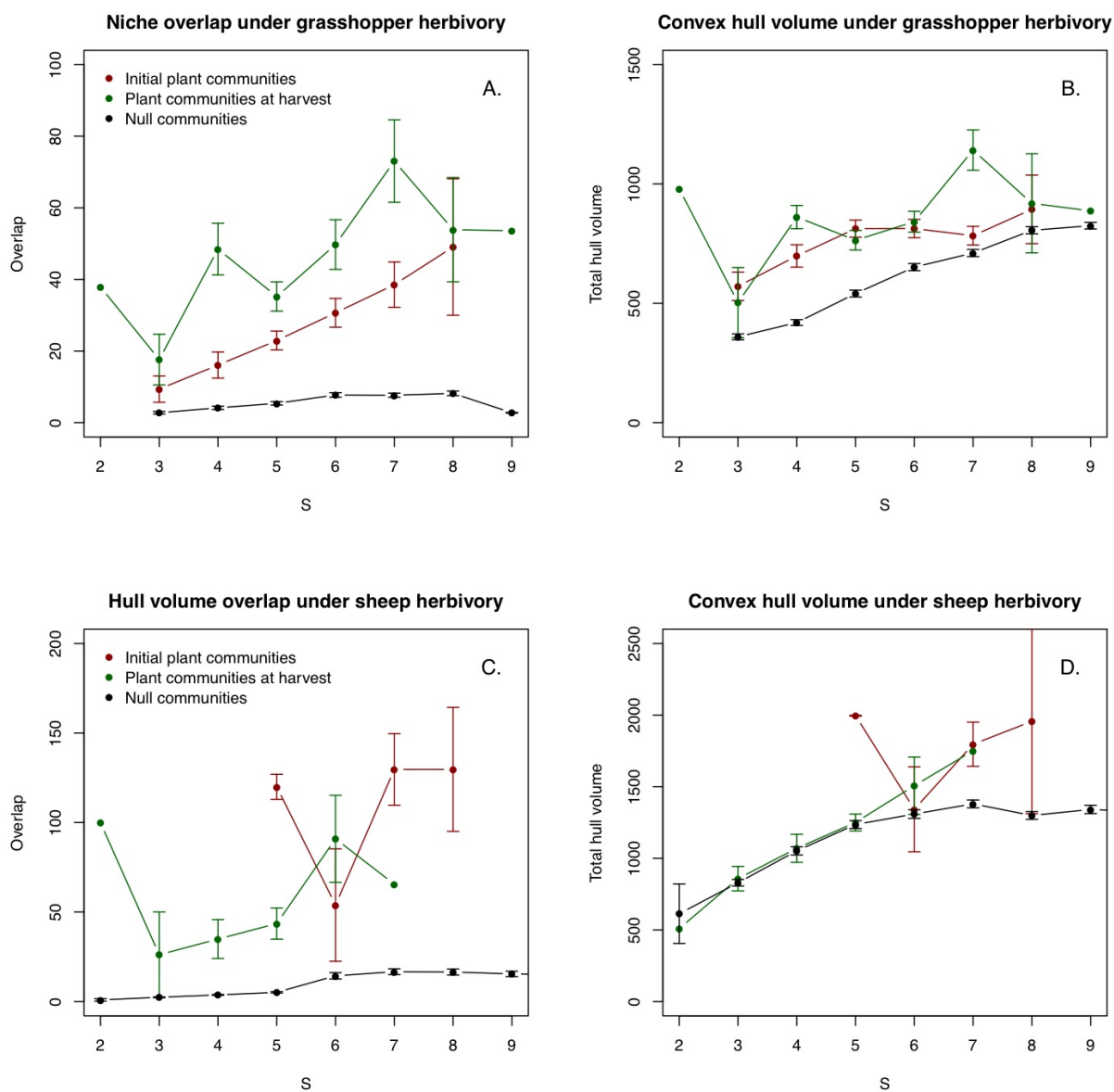
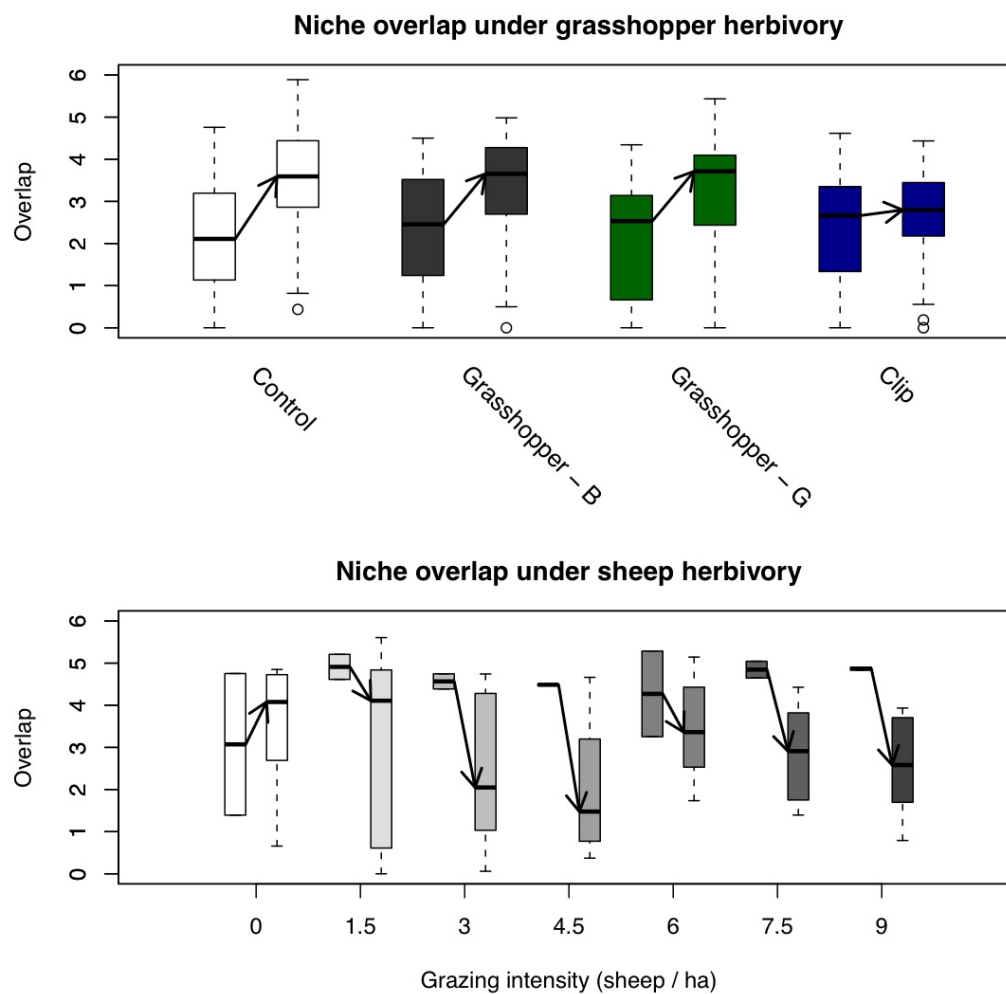


Figure 3.3. Experimental treatments do not alter niche overlap.



## **CHAPTER 4. FUNCTIONAL AND PHYLOGENETIC DIVERSITY AS PREDICTORS OF BIODIVERSITY-ECOSYSTEM FUNCTION RELATIONSHIPS**

### **Summary**

How closely does variability in ecologically-important traits reflect evolutionary divergence? The use of phylogenetic diversity (PD) to predict biodiversity effects on ecosystem functioning, and more generally the use of phylogenetic information in community ecology, depends in part on the answer to this question. However, comparisons of the predictive power of phylogenetic and functional diversity have not been conducted across a range of experiments. I addressed this question in 29 grassland plant experiments, where detailed trait data are available for many species. Functional trait variation was only partially related to phylogenetic distances between species, and the resulting FD values therefore correlate only partially with PD. Despite these differences, FD and PD predicted biodiversity effects across all experiments with similar strength, including in subsets excluding plots with legumes and focusing on fertilization experiments. Two- and three-trait combinations of the five traits used here (percent leaf nitrogen, height, specific root length, leaf mass per unit area, and N-fixation) resulted in the FD values with the greatest predictive power. Both PD and FD can be valuable predictors of the effect of biodiversity on ecosystem functioning.

## Introduction

Substantial experimental evidence exists for the positive influence of biodiversity on ecosystem functioning, especially in grasslands, with a focus on aboveground plant biomass production (Balvanera *et al.* 2006; Duffy 2009). However, which facets of biodiversity most strongly influence ecosystem functioning remains a subject of debate. Recent studies have suggested that phylogenetic diversity (PD, the distinct evolutionary history in a community) can be used as a proxy for these measures of functional diversity (FD, the functional trait distinctiveness in a community); this relationship between PD and FD is premised on the reasonable assumption that evolutionary diversification has generated trait diversification, which in turn may result in greater niche complementarity. This theory has been supported by a meta-analysis of biodiversity-ecosystem functioning studies, finding that phylogenetic diversity (PD) predicted plant biomass accumulation stronger than species richness or functional group richness (Cadotte *et al.* 2008).

Two issues arise in the use of phylogenetic diversity to predict ecosystem functioning, one important to community ecology in general and one specific to grassland biodiversity-ecosystem function research. First, the use of PD to predict ecosystem function assumes phylogeny represents functional differences relevant to a particular ecosystem function (Maherali & Klironomos 2007). This assumption will hold if there is a strong phylogenetic signal in the traits important for determining ecosystem functioning, or in other words that phylogenetic niche conservatism is high for the traits driving community interactions, an assumption central to much recent work at the intersection of evolutionary biology and community ecology (e.g., Cavender-Bares *et al.* 2009). However, while ample evidence for this premise exists for certain traits (e.g.,

wood density, Chave et al. 2006), a recent study found little correlation between changes in mammal body size variation and changes in phylogenetic diversity (Fritz & Purvis 2010), and phylogeny does not always influence competition (Cahill et al. 2008) or niche structure (Silvertown et al. 2006) in plants. Among the traits that drive grassland plant biomass accumulation, coevolved relationships between N-fixing bacteria or with pathogens exhibit strong phylogenetic signal, but such a signal cannot be assumed for all traits. Directly testing for phylogenetic signal in functional trait variation in the context of ecosystem functioning is crucial for determining whether PD can be an effective proxy for FD.

Second, since knowledge of which traits are important to ecosystem functioning and access to high-quality trait data are lacking for most species and ecosystem functions of interest, PD would be quite valuable as a proxy for FD. Grassland biodiversity-ecosystem functioning experiments represent the best case for using plant traits to predict aboveground biomass production. Data on grassland plant ecophysiology and life history are copious, although rarely compiled. Research in grassland communities has underscored the importance of leaf traits such as leaf mass per unit area (Garnier et al. 2004) and leaf percent nitrogen (Kahmen et al. 2006), belowground traits such as root thickness (Craine et al. 2002) and nitrogen fixation (Lee et al. 2003), and whole-plant traits such as height (Díaz *et al.* 2007) in controlling ecosystem processes. Thus, FD and PD should be directly compared in predicting biodiversity effects, and how functional differences map onto phylogenies should be examined.



## Methods

I compiled data from 29 experiments with 1,721 polycultures and 174 species from 11 publications (Naeem *et al.* 1996; Tilman *et al.* 1996; Tilman *et al.* 1997; Naeem *et al.* 1999; Dukes 2001; Reich *et al.* 2001; Fridley 2002; Fridley 2003; Dimitrakopoulos & Schmid 2004; Spehn *et al.* 2005; Lanta & Lepš 2006). For each polyculture, I calculated phylogenetic diversity (PD), functional diversity (FD), species richness (S), and functional group richness (FGR). For the latter, I followed Cadotte *et al.* in assigning species to one of five groups: Nitrogen fixers, woody species, C3 grasses, C4 grasses, and nonnitrogen-fixing forbs.

### *Phylogenetic and Functional Diversity*

I calculated PD from the molecular phylogeny of Cadotte *et al.* (2008), which covered 110 of the species in the meta-analysis, using data for congeners in several cases. In addition, I also calculated PD from a phylogeny extracted from the supertree of Davies *et al.* (2004) using Phylomatic (Webb & Donoghue 2005, <http://www.phylodiversity.net>), which covered all 121 of the species in the meta-analysis, but with much less phylogenetic resolution. I used the phylogenetic diversity measure PD used by Cadotte *et al.*, which is the sum of the branch lengths for the species present in a community. This metric is based on the PD developed by Faith (1992), which differs from the present index by always including the root node. For the supertree-based phylogeny, branch lengths were based on the angiosperm-wide divergence dates, interpolated for undated nodes using the branch length adjustment algorithm in the software Phylocom (Webb *et al.* 2008). The PD values calculated from these two

phylogenies were highly correlated ( $r^2 = 0.964$ ), but yielded different model comparison results.

Calculating functional diversity requires several key decisions. I used the metric FD proposed by Petchey & Gaston (2002) because it exactly parallels PD, accommodates a variety of data types, and has been widely applied as a measure of functional diversity. Which and how many traits are used to calculate FD are the most critical questions in this analysis. I selected a small number of traits known to be important for biomass production in grasslands and for which data are widely available. These traits were leaf mass per unit area (LMA), plant height, leaf percent nitrogen (%N), specific root length (SRL, a measure of root thickness), and whether the plant supports root nodules capable of biological nitrogen fixation (Table 2). Continuous data were rescaled to center on 0 with an s.d. of 1. I calculated FD values from all 26 combinations of 2-5 traits for each polyculture, focusing the results on the FD with the best predictive power for a given analysis and the FD with all five traits.

FD requires calculating the multivariate distance between each pair of species based on their functional traits; I used Gower distances to accommodate both the continuous (LMA, N, height, SRL) and binary data (N-fixation) (Podani & Schmera 2006). Clustering was performed using the unweighted pair group method with arithmetic means, which gave the highest cophenetic correlation with the original trait distances (0.89) of many clustering algorithms. Trait data came from individual studies (e.g., Craine et al. 2001), published compilations (de Faria *et al.* 1989; Wright *et al.* 2004), the LEDA database (Kleyer et al. 2008), reference texts (Grime *et al.* 1988; Gleason & Cronquist 1991), and unpublished data compilations (D. Bunker).

## *Analysis*

For each polyculture, I calculated the net biodiversity effect on aboveground biomass production as the log ratio of the biomass in polyculture ( $y_p$ ) to the mean biomass of the constituent species grown in monoculture ( $y_{\bar{m}}$ ):  $LR_{mean} = \ln(y_p / y_{\bar{m}})$  (Cardinale et al. 2006). Since not all experiments had every species grown in monoculture,  $LR_{mean}$  could only be calculated for 1,433 of the polycultures (see Table S1 for data summary). When using PD values calculated from the molecular phylogeny, additional plots were excluded because this phylogeny did not cover all species, yielding 1,088 plots.

I assessed the relative importance of each diversity metric in predicting  $LR_{mean}$  using single-variable mixed effects models. I further assessed the predictive power of the best functional diversity metric in combination with phylogenetic diversity, to test whether the two types of diversity in combination would yield greater predictive power than either alone. Model parameters were estimated by restricted likelihood estimation, and compared by Akaike weights. Goodness-of-fit for these models was assessed by  $R^2$  of observed and model-fitted  $LR_{mean}$  values. Fourteen outliers identified from a Bonferroni 2-sided test on Studentized residuals were removed. I examined two subsets of the data set, separately examining the diversity metrics in experimental units that 1) did not include legumes, and 2) were experimentally fertilized. Legume presence is an important factor in many grassland biodiversity experiments (e.g., Marquard et al. 2009), and biodiversity-ecosystem function relationships can vary depending on soil fertility

conditions (Reich *et al.* 2001; Lanta & Leps 2007), so these subsets allowed us to compare these different aspects of biodiversity under different conditions.

In order to account for the complex covariations among the alternative measures of biodiversity (Fig. 2, Fig. S3), I also employed structural equation modeling (SEM). Both the PD and FD metrics used here are highly dependent on species richness. The models tested reflect this dependency, and are constructed to test how PD and/or FD mediate the effect of species richness on the biodiversity effect ( $LR_{mean}$ ). Alternative pathways included direct effects of S on the ecosystem function, the inclusion of functional group richness, and correlations between FD and PD (Fig. S2). SEMs were implemented using the R package *sem* (Fox 2006).

I assessed the phylogenetic signal in the functional traits at three levels. First, I compared the relationship between PD and FD. Second, I compared the distances between species based on functional traits with distances based on phylogeny; these distances are the foundation for the diversity metrics. I tested the degree of phylogenetic signal in each trait using the K statistic (Blomberg *et al.* 2003), as implemented in the R package *picante* (Kembel *et al.* 2010). All analyses used the statistical programming software R 2.11.0 (<http://www.r-project.org>).

## Results

PD and FD had similar predictive power for biodiversity effects in all cases. From the mixed effects model comparison, PD was the best predictor of the biodiversity effects on aboveground biomass, followed closely by the combination of PD and the FD calculated from leaf %N, mean plant height, and N-fixation ability ( $FD_{N, Height, N-fixation}$ ),

and then by  $FD_{N, \text{Height}, N\text{-fixation}}$  alone. In the most inclusive comparison, using 1,419 plots and the PD based on the angiosperm supertree,  $FD_{N, \text{Height}, N\text{-fixation}}$  was the best predictor of the effect of plant biodiversity on aboveground biomass production, although PD had similar predictive power (Table 1). When examining only plots that did not include legumes, PD was the best predictor, followed by  $FD_{N, \text{Height}}$ . Examining only experiments where N fertilizer was added, PD was a weaker predictor than FD across all experiments, with  $FD_{\text{Height}, N\text{-fixation}}$  as the best predictor overall. In every case, FGR was the weakest predictor of biodiversity effects. Combining PD with the best FD resulted in greater variance explained for the biodiversity effect on aboveground biomass, but was not the most parsimonious model in any case.

Despite the similar power for FD and PD to predict biodiversity effects in grassland experiments, the relationship between the indices results almost entirely from the correlation of each with S. While PD increases nearly linearly with S, a large range of FD values was found at all levels of S (Fig. 2, Fig. S3), resulting in a modest relationship between FD and PD (e.g.,  $FD_{N, \text{Height}, N\text{-fixation}}$  and PD,  $r^2 = 0.237$ ). The relationship is much reduced when the S effect is removed (residuals of  $FD_{N, \text{Height}, N\text{-fixation}}$  and PD against S,  $r^2 = 0.02$ ), indicating correspondence between FD and PD is not a given at a particular level of species richness (Fig. S3).

Comparison of competing structural equation models demonstrated that for all subsets of the data, the best-fit model required including both PD and FD as predictors of the biodiversity effect. Including the correlation between PD and FD improved the model fit for various subsets of the data (excluding legume-containing plots or unfertilized plots), but not all (Table 3). However, in all cases when the correlation was included, the

value was small (e.g., Fig. 3). The strength of the predictive power of PD and FD in the SEMs largely corroborated the results of the linear mixed models.

Directly examining the phylogenetic signal in trait variation, significant phylogenetic signal was only detected for N-fixing ability (Table 4, Figure S1). When using the angiosperm supertree, with a complete coverage of species but only genus-level resolution, significant phylogenetic signal was detected for LMA, height, and N-fixing ability, indicating that close relatives were more likely to have similar trait values than would be expected by chance.

## Discussion

Our analyses demonstrate that measures of functional and phylogenetic diversity have similar abilities to predict biodiversity effects; functional group richness has the weakest predictive power in nearly all cases. The similar predictive power of FD and PD is surprising because the two indices are based on mostly different information, ecophysiological traits for FD versus time since evolutionary divergence for PD. There is evidence for phylogenetic signal in N-fixation, unsurprisingly, but the diversity metrics summarizing the functional and phylogenetic information do not correlate after the effect of species richness is removed, and SEMs demonstrated small or zero correlation between the two diversity metrics when species richness was also included.

The lack of correlation between FD and PD values for communities of a given species richness suggests that while the traits used in the FD calculations are important, additional axes of trait variation are captured in PD. These un-measured traits may include pathogen tolerance (Petermann et al. 2008) or other coevolutionary relationships,

and seem to be important in determining grassland ecosystem functioning. PD potentially captures all such additional axes, but is not informative for identifying what they might be. Identifying the traits that drive ecosystem functioning will spur better understanding of the consequences of species loss and the mechanisms driving ecosystem processes, such as niche complementarity and the selection effect, and will clarify how evolutionary history can be a good proxy for trait measurements. I found that variation in leaf %N, height and N-fixation were consistently the most important traits for predicting biodiversity effects. Leaf N concentration relates to resource acquisition strategy, while height relates to partitioning of light resources in grasslands (Grime 2001). Differentiation in height and LMA was partially driven by phylogenetic relationships (Table 4). N-fixation coincides completely with Fabaceae, and is the only trait with an overwhelming phylogenetic signal. However, PD was still an effective predictor of the biodiversity effect even when plots with legumes were excluded (Table 1). Thus, phylogenetic divergence can reflect functional differentiation, but this does not result in diversity metrics that correspond closely at a given level of species richness.

Previous studies have evaluated the performance of different diversity metrics in predicting biodiversity-ecosystem function relationships, notably Petchey et al. (2004b), who demonstrated that FD was a stronger predictor of aboveground biomass production than S or FGR. Notably, Cadotte et al. (2009a) assessed PD, several versions of FD, and other diversity metrics as predictors of the biodiversity effect in one of the studies included in this meta-analysis. They found that FD and PD were weakly correlated, but that PD and combinations of PD and other metrics were always superior predictors of ecosystem functioning. This contrasts with the present results, but their study differed

from the current study because they used a different set of traits, fewer species, and focused on a single biodiversity experiment. These contrasting results highlight the need for a mechanistic understanding of which traits are represented by PD.

Importantly, other studies have found that the traits of the dominant species can be more important than any aggregate measure of functional diversity in determining ecosystem processes (Mokany *et al.* 2008; Griffin *et al.* 2009). This highlights the need for further analyses of how plant traits control ecosystem processes, to partition complementarity from selection effects, which I did not address here. In addition, trait data compilation remains a challenge, with a clear need for a central repository of functional trait data. I suggest that further progress in resolving these issues will require examining for what traits and to what extent evolutionary relationships closely match functional relationships, i.e., the idea that there may be a high degree of phylogenetic niche conservatism in the traits important for ecosystem functioning (Ackerly & Reich 1999).

## **Acknowledgments**

Nicholas Mirotnick, Meha Jain, Mathew Palmer, and Shahid Naeem collaborated in conceiving of this study and writing the results. I thank the authors of the original studies for generously sharing their data, M. Cadotte, B. Cardinale, and T. Oakley for sharing their molecular phylogeny and R code for calculating PD, and B. Schmid and Naeem lab members for constructive feedback.



## Tables and Figures

Table 4.1. Model comparison results of linear mixed models.

Models are compared to predict the log response ratio of biomass production for all plots, including without legumes and fertilized experimental plots. Predictors are ranked by Akaike weight. Comparisons were performed between 26 trait combinations for functional diversity (FD), phylogenetic diversity (PD), species richness (S) and functional group richness (FGR), and a multivariate model combining PD and the best FD. Results are shown from PD based on the molecular phylogeny of Cadotte et al., which covers 110 of the 121 species used in these plots, as well as from PD based the angiosperm supertree. Cadotte et al. created a phylogeny of 145 species, of which 121 are present in plots where  $LR_{\text{mean}}$  can be calculated.  $N$ , number of experimental units in this subset;  $w_i$ , Akaike weights.

Using PD from molecular phylogeny (110 species)				
Subset	$n$	Predictor	$R^2$	$w_i$
All plots	1074	PD	0.196	0.989
		PD+FD <sub>N.Height.N-fix</sub>	0.197	0.01
		FD <sub>N.Height.N-fixation</sub>	0.181	4.8 x10 <sup>-5</sup>
		S	0.177	5.5 x10 <sup>-6</sup>
		FGR	0.17	7.5 x10 <sup>-9</sup>
No legumes	506	PD	0.105	0.48
		FD <sub>N.Height</sub>	0.096	0.146
		PD+FD <sub>N.Height</sub>	0.107	0.064
		S	0.097	0.043
		FGR	0.074	3.3 x 10 <sup>-6</sup>
Fertilized plots	212	FD <sub>Height.N-fixation</sub>	0.172	0.216
		PD	0.186	0.117
		PD+FD <sub>Height.N-fix</sub>	0.188	0.024
		S	0.161	0.002
		FGR	0.123	6.7 x10 <sup>-5</sup>
Using PD from angiosperm supertree (121 species)				
Subset	$n$	Predictor	$R^2$	$w_i$
All plots	1419	FD <sub>N.Height.N-fixation</sub>	0.223	0.907
		PD+FD <sub>N.Height.N-fixation</sub>	0.229	0.003
		PD	0.223	0.002
		S	0.204	2.3 x10 <sup>-8</sup>
		FGR	0.187	2.4 x10 <sup>-16</sup>
No legumes	636	FD <sub>N.Height.SRL</sub>	0.12	0.495
		S	0.11	0.001
		PD	0.123	2.7 x10 <sup>-4</sup>
		PD+FD <sub>N.Height.SRL</sub>	0.125	3.8 x10 <sup>-5</sup>
		FGR	0.078	4.8 x10 <sup>-9</sup>
Fertilized plots	302	FD <sub>Height.N-fixation</sub>	0.221	0.606
		PD	0.22	0.002
		PD+FD <sub>N.Height</sub>	0.233	2.8 x10 <sup>-6</sup>
		S	0.204	2.6 x10 <sup>-7</sup>
		FGR	0.198	3.5 x10 <sup>-10</sup>

Table 4.2. Sources of species mean trait data for the 121 species in this analysis.

Values show median and range of trait data and summarize the binary variable.

<i>Trait</i>	<i>Sources</i>	<i>n</i>	<i>Functional significance</i>	<i>Values</i>
Leaf mass per area (LMA, g / m <sup>2</sup> )	Glopinet (22), LEDA (14), Literature (51)	87	Resource capture rate; decomposition; leaf lifespan	49.0 (21.9-141.3)
Leaf N (% on mass basis)	Glopinet (31), Literature (40)	71	Rate of resource capture	2.5 (0.5-5.2)
Height (cm)	Literature (46), LEDA (52), Grime et al. (4), Gleason & Cronquist (11), USDA (7)	12 0	Light competition strategy; competitive ability	35.0 (8.5-1875)
Specific root length (SRL, g / cm)	Craine et al. 2001	24	Investment belowground, root lifespan	96.6 (22.9-288.4)
N-fixation (binary)	de Faria et al. 1989	12 1	Competitive ability in N-poor soil	0: 103; 1: 18

Table 4.3. Summary of structural equation modeling results.

The best of eight possible models is shown for each subset of the data, using either the molecular phylogeny or angiosperm supertree as the basis for PD. RMSEA: root mean squared error approximation. Models "M3" and "M8" both include PD and FD; M8 includes a correlation term between PD and FD. See Table S2 and Figure S3 for complete results.

Using PD <sub>M</sub> from molecular phylogeny (110 species)						
Subset	<i>n</i>	Model	$\chi^2$	<i>df</i>	<i>P</i>	RMSEA
All plots	1074	M8	3.37	1	0.067	0.047
No legumes	506	M3	3.05	2	0.217	0.030
Fertilized plots	212	M3	0.13	2	0.937	<0.001
Using PD from angiosperm supertree (121 species)						
All plots	1419	M8	6.31	1	0.012	0.061
No legumes	636	M8	3.32	1	0.069	0.060
Fertilized plots	302	M3	2.92	2	0.232	0.039

Table 4.4. Phylogenetic signal in the trait variation.

Using Blomberg's  $K$  statistic.  $n$  = number of species with trait data represented in the given phylogeny. Values in bold are statistically significant.

	Using molecular phylogeny		Using angiosperm supertree	
	$K$	$n$	$K$	$n$
LMA	0.240	64	0.326	87
N	0.268	45	0.343	63
SRL	0.282	11	0.358	24
Height	0.273	82	0.635	120
Nitrogen-fixation	6.197	83	9.017	119

### Figure Legends

Figure 4.1. Phylogenetic diversity (PD) is the best predictor of the effect of biodiversity on aboveground biomass production, compared to functional diversity (FD), species richness (S) and functional group richness (FGR), across 1,074 experimental units from 29 experiments. Net biodiversity effects ( $LR_{mean}$ ) are represented by the log ratio of the aboveground biomass of a polyculture to the mean biomass of the constituent species grown in monoculture. Solid lines show fits of single-variable linear mixed-effects models (Table 1), with goodness-of-fit shown by Akaike weights ( $w_i$ ) and model  $R^2$ . Points represent experimental units, and are semi-transparent.

Figure 4.2. Relationships between the three continuous measures of biodiversity used in this study. Histograms are shown in the diagonal, with  $R^2$  values shown in the bottom panels.

Figure 4.3. Best-fit structural equation model combining S, FD, and PD calculated from the molecular phylogeny ( $\chi^2 = 3.37$ ,  $df = 1$ ,  $P = 0.067$ ). Values give the standardized coefficients for the relationship between 'upstream' and 'downstream' variables; all coefficients are significant. Epsilons represent the error term for downstream variables. See Supplemental Materials for full set of models.

Figure 4.1. Comparison of diversity metrics.

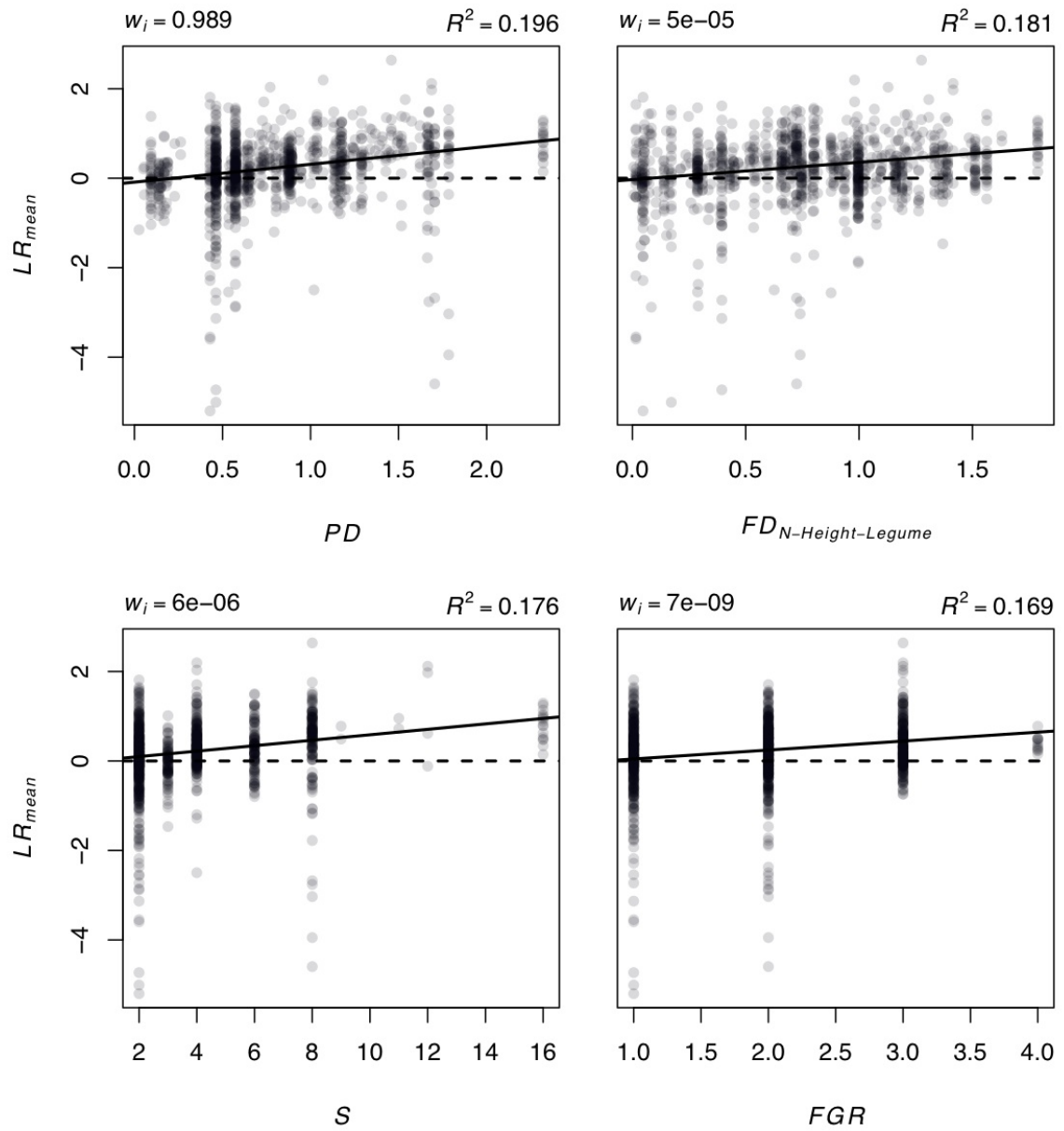


Figure 4.2. Correlations between diversity metrics.

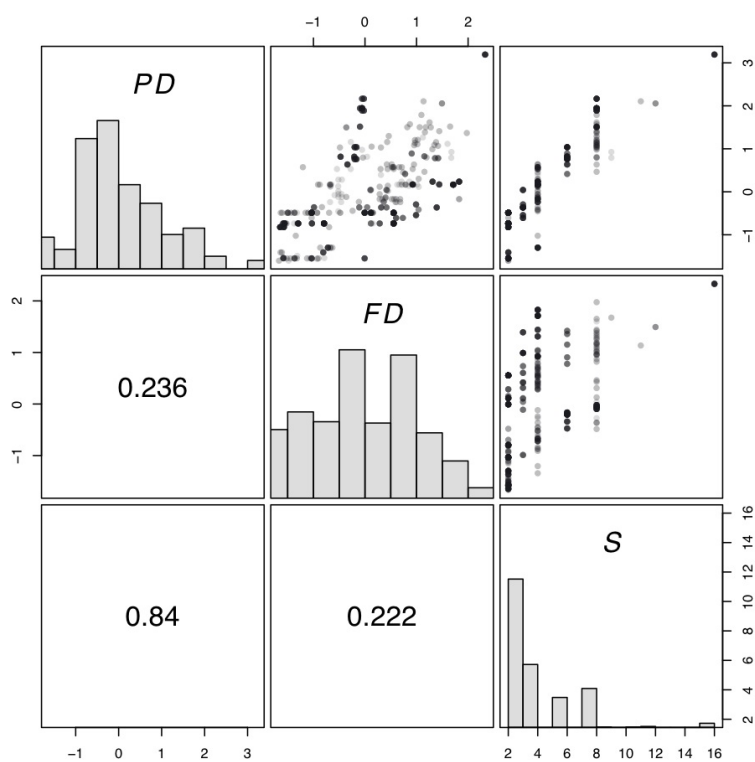
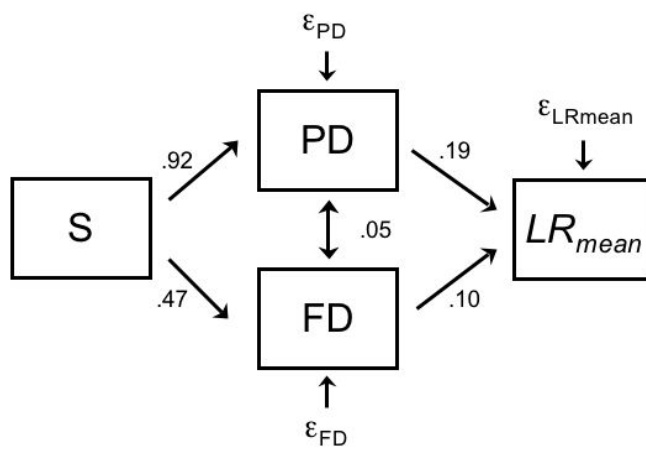


Figure 4.3. Structural equation model.





## CHAPTER 5. SUMMARY

The consequences of biodiversity loss for ecosystem functioning and potentially for the provisioning of ecosystem services has motivated substantial research into the relationship between diversity and ecosystem functioning. The majority of this research effort has been focused within trophic groups, in particular within grassland plant communities, laying the foundation for future progress in two fronts: 1. incorporating multiple trophic levels into biodiversity and ecosystem functioning, and 2. the use of functional traits to investigate community assembly processes and to measure the aspects of diversity most relevant to ecosystems. Both goals have the proximate aim of increasing the realism of research into how diversity loss should be expected to affect ecosystem functioning, and the ultimate aim of refining the link between biodiversity conservation and the provisioning of ecosystem services.

My thesis has broadly addressed the causes and consequences of plant diversity in grassland ecosystems. In particular, I focused on how herbivory shapes plant communities, investigating herbivore behavior and plant strategies to respond to herbivores to better understand factors shaping plant diversity. In parallel, I used approaches based on plant functional traits to look at the balance of abiotic and biotic factors shaping the variation in functional diversity of grassland plant communities in Inner Mongolia. Finally, I examined grassland biodiversity-ecosystem functioning experiments globally, to evaluate which aspects of plant diversity are most relevant to ecosystem functioning.

In Chapter 2, "Foraging behavior of a generalist grasshoppers, *Oedaleus asiaticus*," field observations and a controlled laboratory experiment showed that the feeding preferences of a dominant generalist grasshopper in Inner Mongolia were principally for a palatable, N-rich subdominant C4 plant species. Experimental and observational work demonstrated that silica may be actively used by one dominant plant, the bunchgrass *Leymus chinensis*, in response to either short-term herbivory by grasshoppers or long-term sheep grazing intensity. A co-dominant plant, the needlegrass *Stipa grandis*, appears to have the opposite strategy, with lower silica accumulation under high herbivory, highlighting the potential for the growth-defense tradeoff to exist among dominant plant species. However, further work is clearly necessary to translate variation in antiherbivore defense strategies to ecosystem functioning.

In examining the both the processes shaping community assembly in Chapter 3, over a range of herbivore intensities in these grassland plant communities, I have shown that the signal of environmental filtering dominates the observed communities. This work has additionally created a substantial database of functional trait measurements made at the individual level and detailed plant community surveys, both of which will be contributed to data repositories such as Traitnet (<http://traitnet.ecoinformatics.org>) and Vegbank (<http://www.vegbank.org>).

Extending this work on linking traits to communities, I have shown how newly-developed metrics of biodiversity, based on either differences between species in functional traits or divergences between species in evolutionary history, perform as predictors of ecosystem functioning in Chapter 4. Phylogenetic diversity alone explained a surprisingly high amount of the variance in aboveground biomass production over the

29 experiments studied. The surprise comes from the poor correlation between phylogenetic and functional diversity, at least within a given species richness level. The implication is that there are important features of grassland plants aside from the five traits employed in this meta-analysis, and identifying what those functional axes are presents an intriguing challenge. This challenge can be addressed by taking advantage of large databases of traits, such as in Traitnet, in conjunction with widely-available genetic data to reconstruct evolutionary relationships between species. Testing for the amount of phylogenetic signal in these traits will clarify under what circumstances phylogenetic diversity would be expected to serve as a good proxy for functional diversity in assessing both community assembly and community disassembly. It can be conjectured that traits linked to coevolved relationships, such as plant-herbivore interactions, are more likely to be phylogenetically conserved, and thus provide a good starting point.

### **BEF beyond Western grasslands**

Like a great deal of research in plant community ecology, the majority of the research into grassland biodiversity and ecosystem functioning has occurred in western Europe and North America. Thus, such research has occurred in contexts appropriate for investigating fundamental ecological relationships and applications to restoration, but with limited links to sustainable development or conservation in general (Schwartz *et al.* 2000; Srivastava & Vellend 2005). In order to reach broader generalities about biodiversity-ecosystem functioning in contexts relevant for sustainable development, it is important to include a larger range of study ecosystems. The Inner Asian steppe is the largest grassland in the world, with a diversity of plant and animal life second only to the

African savannahs among grasslands (Wu & Loucks 1992). A significant investment into a biodiversity-ecosystem functioning research platform in Inner Mongolia, China by the Chinese Academy of Sciences has revealed that temporal complementarity in plant populations drives a significant biodiversity-stability relationship in these grasslands (Bai et al. 2004). The grasslands of Inner Mongolia support a population of over 20 million people, and over 90% of them are considered degraded (Jiang et al. 2006). In addition, these grasslands face the twin challenges of desertification and overgrazing (Christensen et al. 2004; Wu et al. 2004; Kang et al. 2007). Therefore, progress made in understanding the factors shaping plant communities in these areas, including the impacts of insect herbivores, and the consequences for changes in plant diversity have the potential to contribute to a more sustainable management of the grasslands in the long term.

## Next steps

The data collected in the course of this dissertation allow several further analyses. From the work in Chapter 2, it is clear that feeding preferences from laboratory studies do not always clearly link to feeding behavior in the field. The small set of plant traits assessed here did not provide strong mechanistic explanations of the feeding preferences. Leaf silica content varied in response to herbivory in opposite ways for each of the two dominant grass species, but a more comprehensive survey of chemical defenses in response to grasshopper herbivory would have been ideal. In particular, assessing leaf total phenolics and total alkaloids would be possible with the samples collected here, in a future study. In addition, responses to herbivory in transcription could be directly assessed using frozen leaf tissue collected from *Leymus chinensis* in the cage experiment,

using microarray technology (Snoeren *et al.* 2007; Leakey *et al.* 2009). Despite high technological hurdles and high cost, the rewards of such analysis could be great, by revealing changes to metabolic pathways directly in response to herbivory; the promise of scaling from genes to ecosystems could be achieved in part with such analysis.

Determining which factors are most important in community assembly also still represents one of the grand challenges in ecology. The work in Chapter 3, showing the strong imprint of environmental filtering regardless of identity or strength of herbivory, presents a challenge to biodiversity-ecosystem functioning research. Niche complementarity has been consistently invoked to explain the positive saturating relationship between species richness and grassland biomass accumulation; why then do communities composed of highly-similar species persist? It is possible that niche complementarity is fairly easy to detect in combinatorial experiments, but plays a smaller role in the assembly of natural systems. Reconciling the insights from community assembly and community disassembly research remains a challenge.

In Chapter 4, I demonstrated that phylogenetic diversity explains a high degree of the variation in the effect of biodiversity on grassland biomass accumulation. Surprisingly, little of the variance in the grassland plant traits most commonly thought to influence aboveground biomass accumulation was explained by the phylogeny, and furthermore the functional and phylogenetic diversity indices related only weakly after the common influence of species richness was removed. Therefore, the search for which traits important for grassland ecosystem functioning do in fact show phylogenetic signal represents an important next step. To date, analyses of phylogenetic signal in plant traits have been limited to species mean values (e.g., Thompson *et al.* 1997; Ackerly & Reich

1999), but advances in integrating multiple levels of variation, from the population to the genus and family level, should provide more powerful tools for such comparative studies.

This thesis represents an incremental step forward in towards increasing the degree of realism in biodiversity-ecosystem functioning research by adopting a multitrophic framework with an emphasis on the effects of herbivores on plant communities, as well as focusing on the use of plant traits to assess community structure and functional diversity. In carrying out this research in the context of the under-studied Inner Asian steppe, this thesis demonstrates the potential for the tool of community ecology to contribute to sustainable rangeland management. While translating the lessons from the basic research here to applications requires much additional work, the potential for applications plant traits, herbivory, and community-level interactions to ecosystem management provides a goal for future work to aspire to.

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## APPENDIX A

### *Supplemental Figures and Tables for Chapter 3: High niche overlap in grassland plant communities irrespective of herbivory*

Table S1: Principal components analysis of plant functional traits. Loadings on the first four principal components.

Trait	PC1	PC2	PC3	PC4
Height	-0.419	-0.303	0.014	-0.340
Longest Leaf	-0.442	-0.128	-0.384	-0.150
Aboveground Weight	-0.332	-0.368	0.431	0.069
Area	-0.268	-0.089	0.639	0.120
LMA	-0.132	-0.451	-0.457	0.400
C	-0.428	0.105	-0.195	-0.208
N	0.274	-0.243	0.049	-0.761
$A_{\max}$	0.326	-0.431	-0.066	-0.113
Conductance	0.254	-0.538	0.059	0.219
S.D.	1.72	1.27	1.06	0.93
Proportion of variance	0.33	0.18	0.13	0.10
Cumulative variance	0.33	0.51	0.64	0.74



Figure S2. Biplot of principal components 1, 2, and 3 of the traits used in the niche overlap analysis. Location of individuals in the ordination space shown in grey, with loadings for each trait represented by arrows.

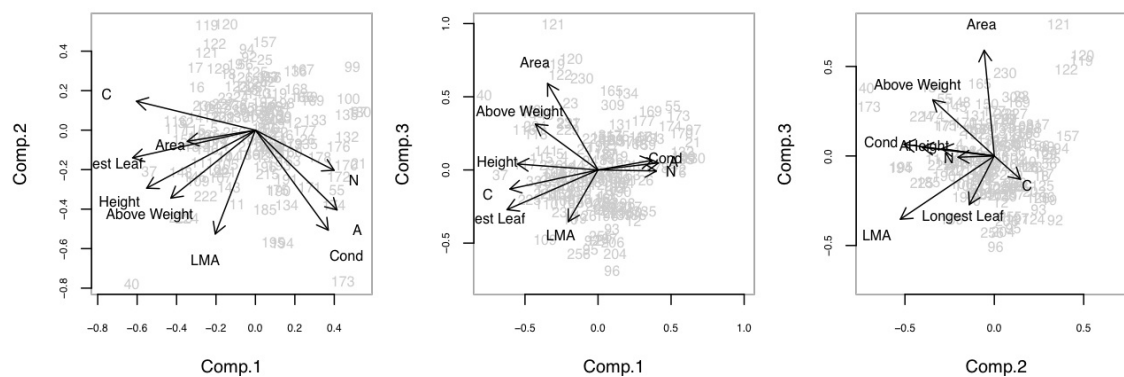
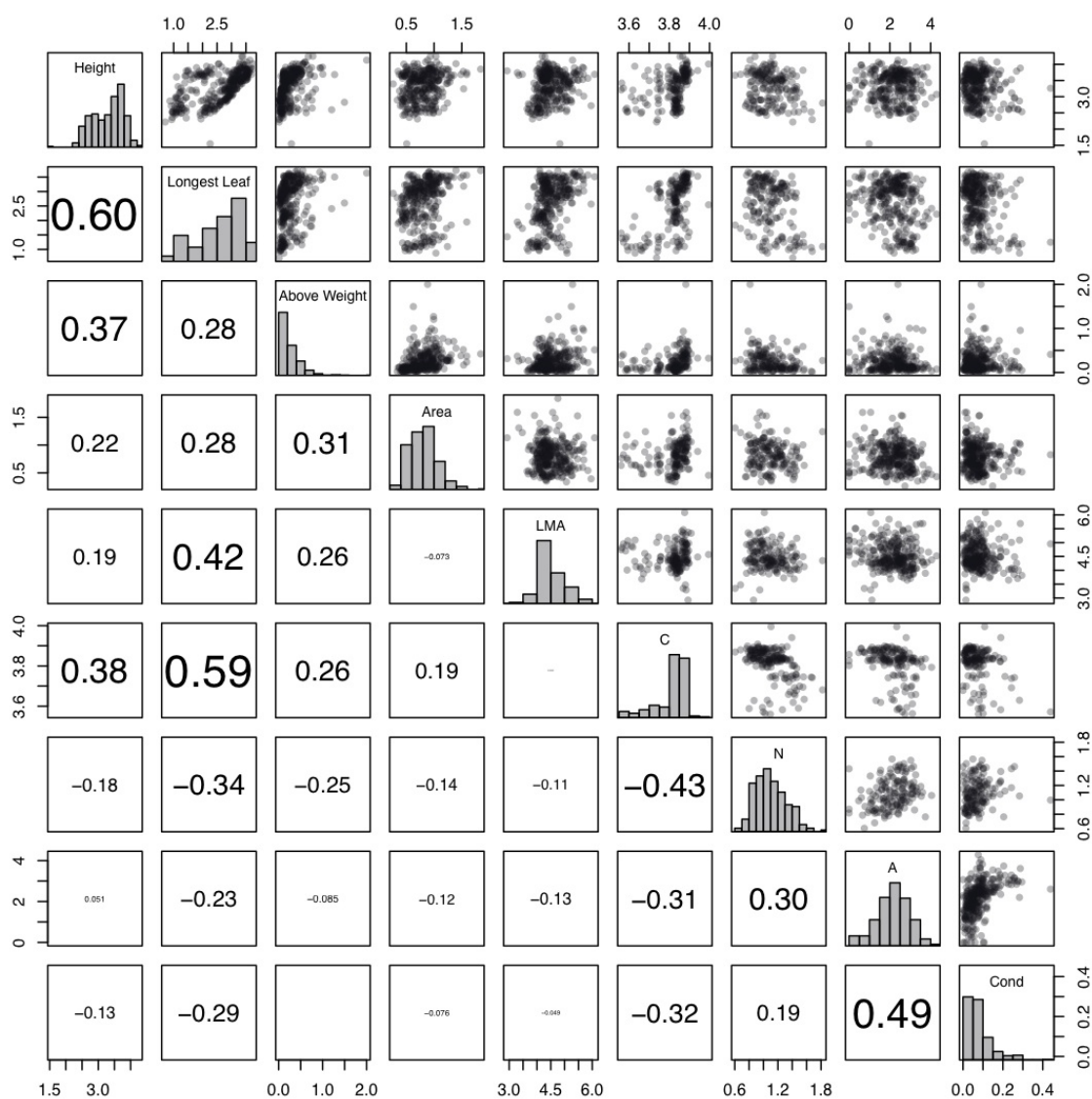


Figure S1. Relationships between plant traits. All traits were measured on all individuals. Pearson product-moment correlations ( $r$ ) between pairs are shown in the bottom panel, with text size proportional to the value of the correlation, and histograms are shown in the diagonal. All trait values were log transformed.



## APPENDIX B

### *Supplemental Figures and Tables for Chapter 4: Functional and phylogenetic diversity as predictors of biodiversity-ecosystem function relationships*

Figure S1. Phylogeny extracted from the angiosperm supertree of Davies et al., showing variation in trait values for the four functional and one taxonomic trait used in this study. White boxes indicate no data were available. Major families for the 121 species used in this study are indicated at right.

Figure S2. Structural equation models tested for combinations of functional diversity (FD), phylogenetic diversity (PD), and functional group richness (FGR) in combination with species richness (S) as predictors of the biodiversity effect on aboveground biomass accumulation ( $LR_{mean}$ ). Models were constructed to represent the effect of PD, FD, or FGR on the biodiversity effect as functions of S, since the indices used here are inherently dependent on S to some extent. That is, the PD and FD indices used here can only remain flat or increase as a species is added to a community. Model 5 shows one of many alternatives where PD and FD do not depend on S; note that this model is consistently the poorest-fitting of the candidate models (Table S2).

Table S1. Sources of grassland biodiversity and aboveground biomass production data.

"Used polycultures" refers to polycultures for which  $LR_{mean}$  could be calculated (1,433 out of 1,593 polycultures).

Study	Experiment	Total polycultures	Used polycultures
BioCON / Reich et al. 2001	+C +N	57	57
BioCON / Reich et al. 2001	+C N	57	57
BioCON / Reich et al. 2001	C +N	58	58
BioCON / Reich et al. 2001	C N	59	59
Biodepth / Spehn et al. 2005	Germany	30	16
Biodepth / Spehn et al. 2005	Greece	6	6
Biodepth / Spehn et al. 2005	Ireland	50	46
Biodepth / Spehn et al. 2005	Portugal	28	23
Biodepth / Spehn et al. 2005	Sheffield	30	30
Biodepth / Spehn et al. 2005	Silwood	32	30
Biodepth / Spehn et al. 2005	Sweden	34	34
Biodepth / Spehn et al. 2005	Switzerland	28	14
Dimitrakopoulos and Schmid 2004	Large pot size	20	20
Dimitrakopoulos and Schmid 2004	Medium pot size	20	20
Dimitrakopoulos and Schmid 2004	Small pot size	20	20
Dukes 2001	-	40	16
Fridley 2002	Amb. nut.	50	50
Fridley 2002	High nut.	49	49
Fridley 2002	Low nut.	47	47
Fridley 2003	High nut./High light	42	42
Fridley 2003	High nut./low light	42	42
Fridley 2003	Low nut./High light	42	42
Fridley 2003	Low nut./low light	42	42
Lanta and Leps 2006	High nut.	58	58
Lanta and Leps 2006	Low nut.	58	58
Naeem et al. 1996	-	330	117
Naeem et al. 1999	-	117	330
Tilman et al. 1996	-	127	6
Tilman et al. 1997	-	148	44

Table S2. Results of structural equation modeling comparisons for the effects of species richness, functional diversity, phylogenetic diversity, and functional group richness as predictors of the biodiversity effect on aboveground biomass accumulation in 29 grassland experiments. The eight candidate models are shown in Fig. S2. BIC: Bayesian information criterion; RMSEA: root mean squared error approximation; CFI: comparative fit index. Note that *P* values indicate whether the model can be rejected as a potential explanation of the covariance in the data set.

Using PD <sub>M</sub> from molecular phylogeny (110 species)						
All: 1,074 plots						
Model	$\chi^2$	<i>df</i>	<i>P</i>	BIC	RMSEA	CFI
M1	35.56	1	<0.001	28.57	0.178	0.9
M2	4.07	1	0.044	-2.92	0.053	0.999
M3	29.5	2	<0.001	15.51	0.112	0.989
M4	26.13	1	<0.001	19.14	0.152	0.99
M5	2333.76	3	<0.001	2312.78	0.845	0.031
M6	967.26	4	<0.001	939.29	0.471	0.721
M7	962.54	3	<0.001	941.57	0.542	0.722
<b>M8</b>	<b>3.37</b>	<b>1</b>	<b>0.067</b>	<b>-3.63</b>	<b>0.047</b>	<b>0.999</b>
No legumes: 506						
M1	4.83	1	0.028	-1.54	0.081	0.991
M2	3.48	1	0.062	-2.89	0.065	0.998
<b>M3</b>	<b>3.05</b>	<b>2</b>	<b>0.217</b>	<b>-9.68</b>	<b>0.03</b>	<b>0.999</b>
M4	1.37	1	0.241	-5	0.025	1
M5	1566.37	3	<0.001	1547.26	0.945	0.013
M6	157.1	4	<0.001	131.62	0.256	0.913
M7	155.41	3	<0.001	136.3	0.295	0.914
M8	1.68	1	0.195	-4.69	0.034	1
Fertilized: 212						
M1	2.44	1	0.118	-2.92	0.083	0.994
M2	0.92	1	0.339	-4.44	<0.001	1
<b>M3</b>	<b>0.13</b>	<b>2</b>	<b>0.937</b>	<b>-10.58</b>	<b>&lt;0.001</b>	<b>1</b>
M4	0.1	1	0.752	-5.26	<0.001	1
M5	638.41	3	<0.001	622.34	1.002	0.048

M6	158.01	4	<0.001	136.58	0.427	0.824
M7	158	3	<0.001	141.93	0.495	0.823
M8	0.03	1	0.862	-5.33	<0.001	1

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Using PD from angiosperm supertree (121 species)

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All: 1,419 plots

Model	$\chi^2$	<i>df</i>	<i>P</i>	BIC	RMSEA	CFI
M1	33.08	1	<0.001	25.81	0.15	0.962
M2	10.86	1	0.001	3.6	0.083	0.994
M3	142.1	2	<0.001	127.57	0.221	0.947
M4	135.79	1	<0.001	128.52	0.307	0.949
M5	2491.83	3	<0.001	2470.03	0.761	0.051
M6	1261.21	4	<0.001	1232.14	0.468	0.697
M7	1253.83	3	<0.001	1232.03	0.54	0.699
<b>M8</b>	<b>6.31</b>	<b>1</b>	<b>0.012</b>	<b>-0.96</b>	<b>0.061</b>	<b>0.998</b>

No legumes: 636

M1	5.12	1	0.024	-1.35	0.08	0.991
M2	10.37	1	0.001	3.89	0.12	0.972
M3	47.74	2	<0.001	34.79	0.188	0.943
M4	44.42	1	<0.001	37.94	0.259	0.946
M5	775.87	3	<0.001	756.44	0.631	0.034
M6	111.59	4	<0.001	85.69	0.204	0.881
M7	108.36	3	<0.001	88.93	0.233	0.884
<b>M8</b>	<b>3.32</b>	<b>1</b>	<b>0.069</b>	<b>-3.16</b>	<b>0.06</b>	<b>0.997</b>

Fertilized: 302

M1	0.04	1	0.841	-5.68	<0.001	1
M2	0	1	0.945	-5.72	<0.001	1
<b>M3</b>	<b>2.92</b>	<b>2</b>	<b>0.232</b>	<b>-8.53</b>	<b>0.039</b>	<b>0.999</b>
M4	0	1	0.986	-5.72	<0.001	1
M5	982.18	3	<0.001	965.01	1.034	0.037
M6	301.64	4	<0.001	278.74	0.494	0.8
M7	297.82	3	<0.001	280.65	0.568	0.801
M8	2.92	1	0.088	-2.8	0.079	0.998

Fig. S1

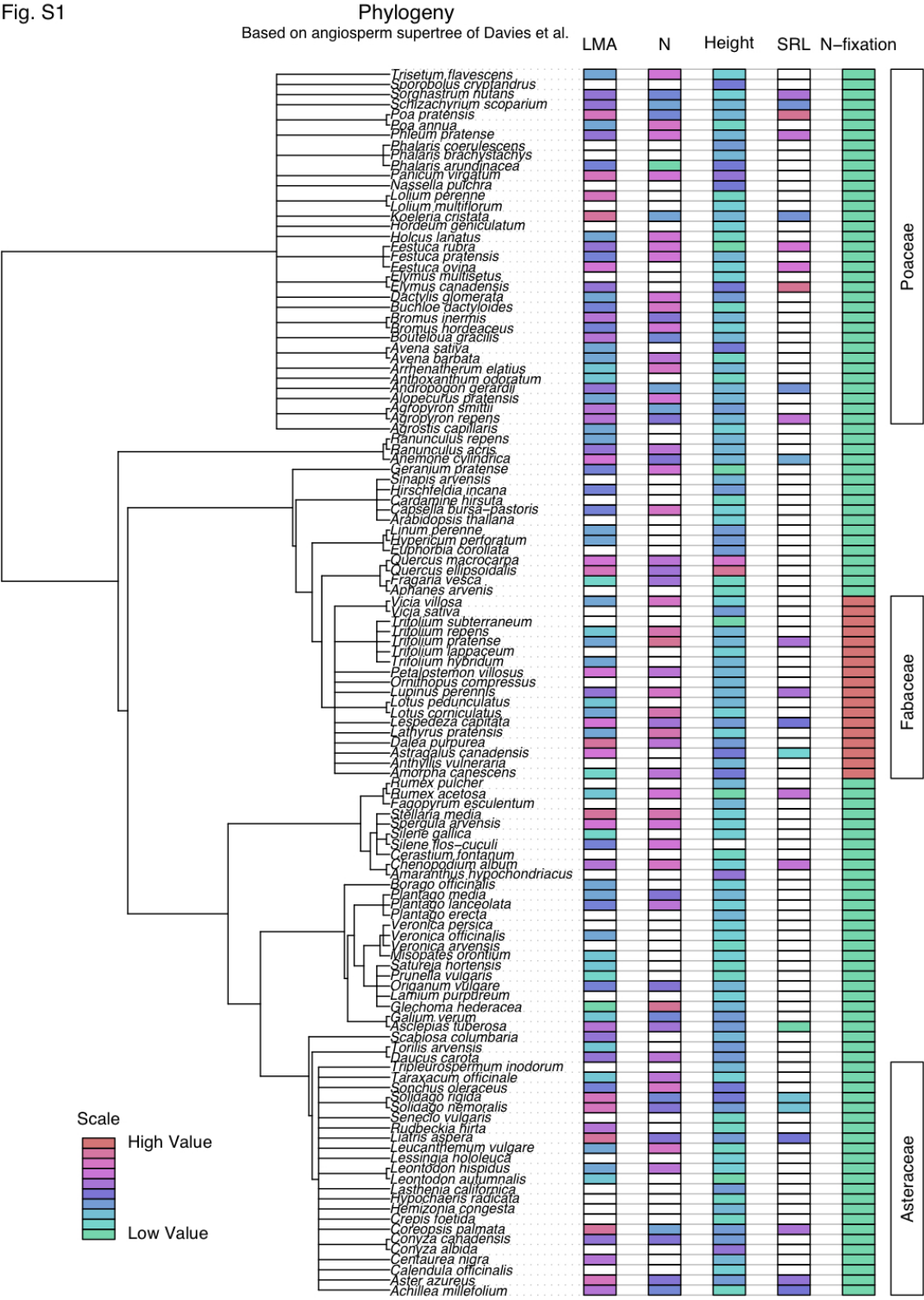


Fig. S2

